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ERRATA AND AUTHORS' EMENDATIONS

Plate 2 (facing page 40), legend, line 3, should read "from top to bottom," instead of "from bottom to top."

Page 185, line 1, should read "60 cc." instead of "66 cc."

Page 235, ftn. 9, should read "Technical Description of the Telfair Citrangequat No. 48005."

Page 235, ftn. 10, should read "Technical Description of the Sinton Citrangequat No. 48050."

Page 237, line 10, should read "Technical Description of Eustis Limequat No. 48798."

Page 237, ftn. 12, should read "Technical Description of Lakeland Limequat No. 48786."

Page 238, ftn. 13, should read "Technical Description of Tavares Limequat No. 48792."

Plate 5 (facing page 238), legend should read "A.—Lakeland Limequat (No. 48786)." "B.—Tavares Limequat (No. 48782)."

Page 273, line 21, should read "M. Frechou, pharmacien" instead of ". . parmacien."

Page 282, line 21, should read "lignified" instead of "ligmified."

Plate 2 (facing page 283), legend, line 2, should read "B.—Same setae shown in A," instead of "B.—Same serae shown in A."

Page 312, 313, should have the legends under figure 9 and figure 10 exchanged (each figure retaining its present number).

Page 316 should have the figure 3 inserted in upper left square on diagram of germination tray between the limbs of the curve.

Page 500, line 28, should read "till inbibition" instead of "til inhibition."

Page 521, line 4, should read "*Aspergillus niger*" instead of "*Aspergillusniger*."

Plate 5, facing page 524, legend, should read "A.–Sample of corn free from internal parasitic fungi" instead of "A.–Sample of corn free from internal paratic fungi."

Plate 1, facing page 582, legend should read "F_2" instead of "F_1" under H.— and I.—

Page 730, lines 21–22 should read "is capable of preventing 60 per cent of the loss of moisture that would occur without mulching" instead of "preserving 60 per cent more moisture in the soil than without mulching."

Page 730, line 16, should read "100 per cent" instead of "1000 per cent."

Page 731: Heading for Table III should read: "Percentage of moisture held by mulches," instead of "Percentage of moisture content of mulches."

Page 740, lines 5–6 should read "capable of preventing 60 per cent of the loss of moisture which would occur from unmulched sand soil," instead of "capable of preserving 60 per cent more moisture in the soil than is retained without mulching."

Plate 3, facing page 760, should read "Plate 4."

Plate 4, facing page 760, should read "Plate 3."

Page 974, Table I, Continued, column 1, should read "July 11" instead of "May 11."

Page 373, last line, should read "(*Puccinia graminis tritici* Erikss. and Henn.)" instead of "(*Puccinia graminis tritici* Pers.)."

Pages 622 and 623, Tables XXVII and XXVIII, head "INFESTED SOIL" should stand within the tables, below the column heads, directly above the tabular matter, in position analogous to that of "CLEAN SOIL" below.

Page 997, line 12, should read "polysaccharids" instead of "polysaccharide."

Page 997, Table I, column 9, line 3, should read "$^{c}1.3$" instead of "1.3."

Page 1001, Table II, column 4, line 11, should read "23.88" instead of "28.88."

Page 1001, ftn., should read "[18]" instead of "[8]."

JOURNAL OF AGRICULTURAL RESEARCH

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A PHYTOPHTHORA FOOTROT OF RHUBARB[1]

By GEORGE H. GODFREY

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INTRODUCTION

Rhubarb footrot is a parasitic fungous disease which manifests itself by a wilting of the leaves, followed shortly after by their collapse at the base of the petioles and the rapid death and decay of the whole plant. It is caused by a species of Phytophthora heretofore unreported in connection with this crop. The disease was first brought to the writer's attention in July, 1917, when a report was received in the Office of Cotton, Truck, and Forage Crop Disease Investigations, of the Bureau of Plant Industry, of a disease of rhubarb in a garden in Brookland, D. C., which was killing out the rhubarb plants. Soon afterwards other reports from different points in the District were received. A survey of the environs of Washington showed that the trouble was rather widespread.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Inasmuch as this disease has not yet been reported on rhubarb and is not widely known among the plant pathologists of the country, only a very incomplete account of its distribution can be given at this time. Furthermore, some of the citations given are necessarily based upon inaccurate identifications, as will be seen, and must serve rather as an indication of localities where the disease may be found than as reliable data on distribution. Owing to a change of duties it has become necessary to submit for publication results as they stand without carrying to a satisfactory conclusion some of the doubtful phases of the investigation.

The disease was observed repeatedly by the writer during 1917 and subsequently in various gardens in the District of Columbia and in neighboring Maryland and Virginia. One newly planted field was seen that was almost entirely killed out. Numerous reports of the dying out of rhubarb plants in this same locality, particularly in small gardens, have also been received, and from the descriptions given, either orally or in writing, it would seem that the cause has been the same as in the cases personally studied. Letters and diseased specimens also were received from more distant points. On September 5, 1917, a specimen was received from Harrisburg, Pa., which was similar in appearance to the Phytophthora footrot familiar to the writer, and which was found to contain in affected parts hyphae of Phytophthora. While the fungus

[1]Accepted for publication July 2, 1921.

was not isolated, it was found in mixed culture to belong to this genus. In November of the same year a similar specimen was received from Amelia Court House, Va.

These several occurrences of the trouble in 1917 led to an examination of the card records of diseased rhubarb specimens and of correspondence received by the Office of Cotton, Truck, and Forage Crop Disease Investigations in previous years. Many cards were found upon which the descriptions recorded compared favorably with that of the disease caused by this Phytophthora. On March 24, 1913, a specimen was received from Bowling Green, Ky., which consisted of "decay of underground stems." On January 24, 1914, a specimen of what was probably forced rhubarb was received from Parkersburg, W. Va. It was called a "crown rot" and described as follows:

> Tissue at base of leaves soft and decayed, decay extending into root; bacteria abundant.

Reference to the paragraph in this paper headed "Description of the Disease" will show that abundance of bacteria is characteristic of the later stages of footrot rather than indicative that bacteria are in any sense primary. On July 31, 1914, a specimen from Mount Rainier, Md., was described as a "'crown rot' . . . tissue full of bacteria." On September 2, 1915, specimens from Richmond, Va., were "affected with a basal stem decay." On August 3, 1916, a specimen "in bad condition" was received in the office from a garden in Washington, D. C. In January, 1920, a letter was received from Cobden, Ill., telling of the occurrence of a disease in rhubarb fields that had killed out a large number of plants. The statement was made that replantings made in the spring of 1919 into hills where rhubarb had died the year previously were killed by the disease that same summer. In the summer of 1921 specimens sent from St. Louis, Mo., showed typical symptoms and the presence of a Phycomycete, probably Phytophthora, in the roots. In addition, reports of rhubarb diseases with no particular clue as to their identity were received from time to time from Kentucky, Tennessee, Missouri, and Idaho.

The earliest report of which the author has been able to find any record was contained in a letter dated July 20, 1902, from Prof. William Stuart, then at Purdue University, La Fayette, Ind., in which he described a disease of rhubarb similar to the one under investigation. Specimens accompanied the letter, but the correspondence shows that they were so much decayed that it was impossible to determine definitely the nature of the trouble.[2]

Beach (*2*),[3] who has made the only mention of such a disease to be found in the literature, reports in his paper and by letter that in the summer of 1920 a rhubarb crownrot due to Phytophthora was prevalent in Philadelphia County, Pa., in one case having killed nearly half the stand in an acre field. As will be shown later in this paper, however, his disease is not identical with that investigated by the writer, the causal organisms being distinctly different.

This fact complicates the matter of distribution considerably, inasmuch as the two diseases appear to be very similar if not indistinguishable. The other organism, or one very close to it, has also been received

[2] An extract from the letter follows: "The rhubarb plantation from which the plant sent you was taken is quite badly affected. The gross characters of the disease in affected plants is the wilting of a leaf or two until finally all are affected, when the crown turns dark and decays. The decay of the crown terminates the existence of the plant. The owner of the rhubarb bed said that he replaced all those that had died the year previous, only to find that the newly set plants soon became affected and succumbed to the disease."

[3] Reference is made by number (italic) to "Literature cited," p. 24–26.

from Ithaca, N. Y., where it was isolated from rhubarb in two separate gardens. It is evident, therefore, that the data here given on distribution may have to do with the disease caused by either one of the organisms, except in those cases in which the specific organism is clearly noted. It is hoped that it will be possible, after another season's observations, to distinguish between the diseases under field conditions. Mr. Beach has much additional data in this connection which will probably be published soon in a bulletin of the Pennsylvania Agricultural Experiment Station.[4] His paper is an important and valuable contribution to this and other phases of the rhubarb crownrot problems.

It is not possible to make any estimate of total loss at this time. Since rhubarb is rather extensively grown on a commercial scale and the disease is widespread, it will have to suffice to say that the footrot is of considerable economic importance.

DESCRIPTION OF THE DISEASE

The first outward manifestation of Phytophthora footrot is a sudden wilting of the foliage, usually of one or two leaves only. The base of the petiole, or leaf stalk, is the point affected. Here a distinct lesion is found, slightly sunken, sometimes not colored, and sometimes in sharp contrast with the pink or light green of the normal petiole because of its brown color. A leaf that has reached the wilting stage is shown in Plate 1, A. The lesion has practically girdled the base of the stem. This area advances rapidly, and in less than 24 hours the stem may collapse at this point, allowing the leaf to fall to the ground. Leaf stalks with the disease slightly farther advanced are to be seen in Plate 2. The tissue is killed so quickly that secondary organisms, particularly bacteria, gain entrance almost immediately. These contribute to the rapid decomposition of the fallen leaf, turning it quickly into a putrid mass, especially at the base, as shown in Plate 2. This factor makes it difficult to isolate the causal organism. Fungi as well as bacteria play a leading part in the further destruction of the leaf. A species of Colletotrichum, probably the same one treated by Stevens (*24*) and considered by him to be *Colletotrichum crumpens* Sacc., is found in great abundance at times on decumbent rhubarb leaves and petioles in early and late stages of decomposition. Plate 3, B, illustrates a leaf petiole recently fallen with Colletotrichum appearing along its entire length. Plate 3, A, shows a leaf entirely covered with this fungus in a fruiting condition.

In weather favorable to the disease the first symptoms are followed in a day or two by the infection and collapse of other leaf stalks in the plant, as shown in Plate 4, A. Finally, all the leaves drop, leaving the plant entirely dead (Pl. 4, B). Meanwhile other plants in the field are similarly affected and many of them are killed, so that the field may show large vacant areas.

A study of many plants in different stages of infection showed that the fungus advances into the root from affected leaf stalks, causing a brown decay. Thence it spreads rapidly through the upper portion of the root, killing the other leaves and leaf buds. Plate 5, A, a section through a recently killed plant, shows the advancing edge of decay due to the causal organism. At the points marked (X) the fungus was found and identified as Phytophthora. Plate 5, B, shows clearly how the fungus,

[4] This bulletin has since appeared: BEACH, W. S. THE CROWN ROT OF RHUBARB CAUSED BY PHYTOPHTHORA CACTORUM. Pa. Agr. Exp. Sta. Bul. 174, 28 p., 25 fig. 1922. Bibliography, p. 27–28.

entering the root from one side, spreads and involves the whole plant. The remaining stalks are doomed to quick collapse, for the fungus has advanced from the other side of the plant, which has already been killed and has penetrated the upper portion of the roots feeding this side. Within a few hours these stalks would present the appearance shown in Plate 4, B.

This disease should not be confused with one very commonly found in rhubarb fields during the summer months, which consists of a dry necrosis of the base of the petiole and its consequent partition from the plant. The latter condition is pictured in Plate 3, C. It is in no way related to the Phytophthora disease, even though stalks so parted from the plant may appear the same (except for more marked yellowing of the leaves), and may go through identical processes of further decomposition and decay.

Rhubarb shows a remarkable power of withstanding and checking further advance of the disease if conditions are not entirely favorable to the causal organism. A change in the weather may prevent the spread of the fungus from a point of primary infection to the rest of the plant. In artificially inoculated plants in which the fungus was known to have entered the roots, however, the writer has seen the disease continue in its destruction even during a period of dry weather.

INFLUENCE OF WEATHER CONDITIONS ON PROGRESS OF THE DISEASE

Marked variation in the prevalence of this disease from year to year was noted. This appears to be due to differences in weather conditions, especially in the rainfall during the period of greatest danger. In 1917 reports of the disease were numerous, and it was found by personal surveys to be rather general. During the next summer very few reports of rhubarb diseases of any kind were received. Field trips into localities that had been badly affected in 1917 did not bring to light any of the disease in its active condition. During the summer of 1919 the writer had no opportunity to investigate its prevalence, but the appearance of the experimental field the next spring indicated rather heavy loss of plants. In 1920 it appeared worse than ever, as was evidenced by numerous oral and written reports and by personal observations. A field at Arlington Experimental Farm, Va., just across the Potomac River from Washington, D. C., was attacked and plant after plant killed by the disease.

A study of the climatological data for Washington, D. C., and vicinity over the summer months of 1917 to 1920, inclusive (*26*), disclosed some interesting facts with regard to rainfall. The total precipitation for June, July, and August, 1917, was 16.43 inches; for the same period in 1918, when the disease was not to be found at all, it was 7.73 inches, or less than half that of the previous year; for 1919, 13.65 inches; and for 1920, 15.21 inches. It is a prolonged period of continuously wet weather, however, rather than a high total precipitation, that is favorable to the development of the downy mildews in general. In this respect, particularly, the weather records show an interesting correlation with the prevalence of the rhubarb disease. In July, 1917, two prolonged periods of daily rainfall and cloudy weather occurred. During the first, from July 8 to July 11, an average of 0.36 inch fell every day, followed by several cloudy days. Again, on July 22, 1917, 0.59 inch of rain fell, followed by two cloudy days (49 and 46 per cent of possible sunshine), and on the next

day 3.27 inches, followed by two cloudy days. During either period conditions undoubtedly favored the development of a downy mildew. During the summer of 1918 no such period of prolonged wet weather occurred. In 1920, when the next observations on the disease were made, several wet periods occurred, the longest of which was from July 15 to July 23, every day being listed as cloudy. In fact, only one day, the 19th, showed as high as 40 per cent possible sunshine. During these nine days 5.6 inches of rain fell. It was during the latter part of the period that the rhubarb disease appeared at its height.

The extensive occurrence of rhubarb footrot only during such weather conditions agrees with the observations of other workers on other diseases of the same type. Clinton wrote in 1905 (6):

> The prevalence of most parasitic fungi is largely influenced by the character of the weather, particularly in regard to moisture. This is especially true of the downy mildews. An abundance of cloudy or rainy weather at certain periods of the year determines whether or not these troubles will be injurious.

CAUSAL ORGANISM

ISOLATION

When investigations on the disease were started, several organisms had to be isolated and studied before the true cause was discovered. Prominent among these was *Colletotrichum erumpens* Sacc., which has already been mentioned as being almost universally found on decumbent rhubarb stems. This fungus was reported by Stevens (*24*) as the cause of a rhubarb disease in Illinois in 1919. One feature that cast suspicion on it in particular was that it was found fruiting in distinct lesions, such as are shown in Plate 3, B, on freshly fallen rhubarb stems. Furthermore, it appeared to be constantly associated with the footrot; the abundance of infective material on old and dried as well as on freshly fallen leaves would seem to be conducive to such damage as was noted. Single spore isolations were made after considerable difficulty to free them from bacteria, and inoculations were made upon living plants in the greenhouse, under most favorable conditions as regards moisture and temperature. Results were consistently negative. Stems cut and inoculated in a moist chamber became infected, but the symptoms differed essentially from those of the footrot disease. Several bacteria were isolated and used for inoculations without positive results. The finding of a coenocytic fungus in the tissues of fresh material at this time led to a concentration of effort on that and abandonment of further systematic study of the other organisms.

This Phycomycete, after its presence was first noted in newly collected material, was constantly found associated with the typical disease. Repeated attempts were made to isolate it by the tissue fragment method on poured plates of corn meal and other agars. While it grew very readily in most of the media used, it was always accompanied by contaminating bacteria, often even out to the very tips of the growing hyphae. Finally in September, 1917, pure cultures were obtained. On corn meal agar plates the organism, contaminated as it was with bacteria, sometimes made a rather profuse aerial growth. By cutting sheets of sterile agar from a poured plate and laying them one on top of another, little inverted pryamids were built on the under surface of the cover of a Petri dish. These were turned with the cover so as to be directly over the colonies with good aerial growth. In two cases out of several attempts

the fungus grew into contact with these blocks of agar, which were soon after turned again over a clean part of the plate and after several hours' growth had been permitted, were found to be free from all contaminations. These sources were used to inoculate several clean plates, and from them a large number of cultures were made on corn meal, oatmeal, and lima bean agars. Two more strains indentical in character with the first were obtained in this manner, one from a garden and the other from a commercial field, both in the District of Columbia.

PROOF OF PATHOGENICITY

A large number of rhubarb seeds were planted in flower pots in the Washington pathological greenhouses, and about the middle of October, 1917, they were large enough for some preliminary inoculation experiments.

INOCULATION No. 1.—A seedling planted August 31 was inoculated on October 19 by puncturing with a sterile needle a leaf petiole near its base and applying a small bit of a culture from a corn meal agar tube. Five days later, not only the inoculated leaf but all the others on the plant had fallen to the ground. Plate 6, A, shows this plant, together with the control similarly treated except for the application of the fungus, photographed October 26.

INOCULATION No. 2.—On October 19 a similar plant was inoculated by the application of the culture without injury at the base of a leaf stalk. The plant was covered with a bell jar which was removed in 24 hours. This treatment was applied to two plants. On October 24 one of the plants was dead, and the other showed the outer leaves wilted down. The control was perfectly normal.

INOCULATION No. 3.—A plant similar to the others was inoculated as in No. 2, but the bell jar was not applied, moist earth being piled up around the inoculum. At the expiration of five days this plant was dead.

INOCULATION No. 4 was identical in method and results with No 2., the plants being killed and the controls remaining healthy. In inoculation No. 5 the root was injured, and the inoculum applied at this point resulted in its decay and the death of the plant as before. In No. 6 the fungus was applied to the root without injury, and at the end of five days the plant was still healthy. In No. 7 the root was injured as in No. 5, with similar results. In No. 8 the petiole was injured, no bell jar was used, and infection was positive. Controls of No. 5, 6, 7, and 8 remained healthy.

On November 24 an artificially infected seedling inoculated about three days previously and showing typical signs of the disease was removed from the greenhouse and an attempt was made to reisolate the organism. One leaf stalk showed a lesion extending up the stem about ¾ inch and a rot extending into the root from ½ to ¾ inch on one side. In a microscopic mount from the advancing edge of the decay, hyphae of Phytophthora could be seen apparently free from other organisms. Saprophytic fungi, bacteria, and nematodes were abundant at the points of earliest decay. Isolation attempts on rhubarb juice agar and potato agar plus rhubarb juice, made by the tissue fragment method, failed to get the organism into pure culture, though the Phytophthora developed and fruited.

These results were so strongly indicative that the right organism had been found as to justify confining further operations practically to this fungus.

Pathogenicity was completely proved later. Meanwhile a large number of rhubarb roots of standard varieties had been obtained from a seedsman. These were planted, both in a field plot and in flowerpots in the greenhouse, at Arlington Farm. In the greenhouse further inoculations were made on the large plants obtained from roots, and these were almost invariably successful, resulting in the majority of cases in the death of the whole plant and the decay of the root. Inoculations made into both the aerial parts and the roots gave this result. Plantings made in the spring in the commercial grower's field to replace plants that had been killed by the disease the previous season resulted in the decay and death of the new plants. Inoculations into large, vigorous plants in the field at Arlington Farm, made by applying the fungus from pure culture at the base of a stalk where moisture is abundant and growth is rapid, resulted not only in the death of the particular stalk inoculated but in the rapid destruction of the entire plant.

It became necessary to discontinue the investigation of this problem about midsummer, 1918, in order to take up important war emergency work. Except for the maintenance of cultures and occasional observations, it was not until the summer of 1920 that the writer was again able to devote any considerable attention to the problem. Early in July, 1920, the experimental field at Arlington Farm was in vigorous growing condition, and none of the disease was evident. Another series of inoculation experiments was started under these conditions. When the inoculations were made the weather was not altogether favorable for infection, and water and shade were applied artificially, as indicated below. A period of wet weather followed immediately after, however, and this resulted not only in the rapid development of the fungus in plants inoculated but also apparently in its spread to other plants. The experiment is reported in detail in Table I.

TABLE I.—*Inoculations of rhubarb plants with Phytophthora parasitica var. rhei.*

Inoculation No.	Date.	Method.[a]	Part of plant.	Location.		Results.	Remarks.
				Row.	Plant.		
[b] 9	July 6	Culture applied without injury	Center, on young leaves and stalks	I	5	July 9, negative	
10	...do	Zoospores	Young leaves, and petioles	I	5	July 9, positive	Reisolation made.
11	...do	Culture applied without injury	General, at bases of leaf stalks	I	6	July 12, positive	Photographed.
12	...do	Culture applied with puncture of scalpel	General, as above	I	9	July 9, positive	Spreading.
13	July 9	Culture applied with injury	General, in crown, after pulling 3 stalks	III	4	July 12, positive	
14	...do	...do	Local, at bases of particular stalks	II	7	...do	Photographed; reisolation made.
15	...do	...do	...do	II	8	...do	
[c] 16	July 12	Zoospores, without injury	Young leaf	IV	1a	July 16, positive	In spots.
17	...do	Culture applied with injury	Base of stalk	IV	1b	...do	Stalk wilted down.
18	...do	...do	...do	IV	1b	...do	Do.
19	...do	Zoospores applied without injury	Leaf, petiole, and base	IV	1c	...do	In local areas, spreading.
20	...do	...do	...do	IV	1c	...do	Do.
Controls.							
9	July 12	Some injured, others not; marked	Other leaves	IV	2	July 16, negative	No infection.
10	...do	Bordeaux mixture 4-4-50 applied	On special, young leaves	IV	3	...do	No injury from the Bordeaux.

[a] In general, inoculum was applied by transferring directly from the culture tube with a sterile needle. Zoospore suspensions were poured directly from tubes.

[b] The inoculations were made at 3.30 p. m. on a clear, sunny, and windy day. The plants were sprinkled with tap water from a sprinkling can, and newspapers held in place with weights were spread about the plant to provide shade.

[c] On July 12 the sun was shining and a dry wind blowing at the time the inoculations were made. A large wooden box was placed over the entire series and left there till final observations were made as noted.

Inoculations No. 21 to 28 were made similarly, except that the inoculum was applied in each case to the crown at the points from which stalks were pulled. From three to five points were inoculated in each plant. Immediately after the plants were inoculated every other plant was sprayed at its base with 4–4–50 Bordeaux mixture, the intent being to determine whether plants could be protected from infection by this means. Inoculation and spraying were done on July 12. Five days later observations were made and final notes were taken in the spring of 1921. Eventually three of the four plants inoculated and not sprayed succumbed to the disease. Spraying served to prevent infection, for none of the sprayed plants died.

In connection with the motion-picture film illustrating zoospore emergence, mentioned by Godfrey and Harvey (*11*) another inoculation, No. 29, was made on July 20. Three or four stalks were pulled from a large plant and inoculum was applied from a Petri dish culture at the broken stubs in the crown. This inoculation resulted in the spread of the organism to other parts of the plant and the ultimate death of the plant. Plate 6, B, shows this plant photographed on July 26. Plate 4, B, is from a photograph of a plant that became diseased as the result of inoculation 11 (Table I) photographed July 17.

As indicated in the tabulations after inoculations No. 10 and 14, reisolations were made from affected parts. In the case of the leaf inoculated by application of zoospores (No. 10) reisolation was accomplished by cutting out a portion of the base of a large leaf vein that had become infected, washing it for about a minute in mercuric chlorid 1 to 1,000 and then in three changes of sterile water, and applying in pieces broken apart with sterile forceps to an agar plate. This resulted in a pure culture of the same organism as was used in making the inoculation. An isolation was made from the base of the affected stalk in No. 14 in practically the same way. One of the bits gave a pure culture. These cultures were used for successful inoculations in the greenhouse during the following winter. Thus Koch's rules of proof of pathogenicity were completed and this organism was established definitely as the cause of the disease.

Still further inoculations and reisolations were made in the spring of 1921 on some potted rhubarb plants in the greenhouse. At this time an easy method of isolation to free quickly from contaminating organisms was worked out. On the chance that it may be useful to other workers on Phytophthora, this method is here described. It consists simply of inoculating with the mixed culture a good sound apple, according to the methods described completely in connection with another phase of the problem on page 15 of this paper. The Phytophthora quickly outgrew the contaminating bacteria and in about two days was obtained absolutely pure by transferring from the advancing edge of decay in the interior of the apple. Plate 7, B, shows the fungus in pure culture, growing from such transfers of apple tissue, compared with the contaminated culture A, made by transferring from near the point of inoculation.

DESCRIPTION OF THE FUNGUS

So much has been written from time to time of the characters peculiar to members of the genus Phytophthora and of their behavior in culture that an effort will be made here to avoid useless repetition by reference to previous papers wherever possible. An exception will be made of

points upon which emphasis is deemed desirable, and, of course, of any new points that may be brought out.

Morphologically distinct sporangiophores of this fungus have never been observed by the writer in nature. Sporangia have been observed, but always attached to hyphal branches that could not be distinguished by any morphological characters from other aerial hyphae of the fungus. In pure culture this same condition seems to exist. Attempts were made with the use of Van Tieghem cells to produce sporangiophores, as was done by Rosenbaum with several different species of Phytophthora (*21*). In the aerial hyphae that developed, sporangia could be seen on irregularly branched hyphae. In no case were clusters of sporangia seen, such as are pictured by Rosenbaum. Plate 8, D, E, F, and Plate 9, G, H, are camera-lucida drawings of sporangia as found in pure cultures attached to the hyphae.

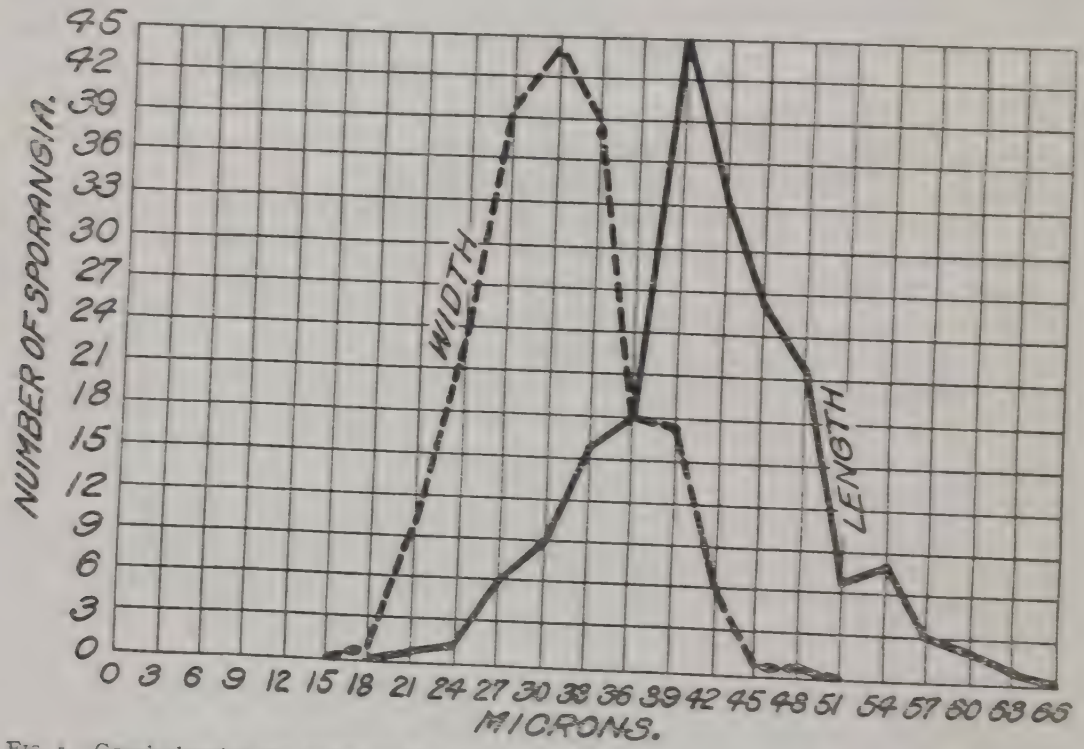

FIG. 1.—Graph showing the variation in length and width of sporangia of *Phytophthora parasitica* var. *rhei*.

Sporangia are variable in size and shape but on an average would be said to be comparatively large, and of the short, ovate type rather than the long, slender kind. Figure 1 shows the results of 200 measurements and shows graphically the sizes of greatest frequency. It will be seen that practically all of them fall within the limits of 33 to 48 μ by 24 to 39 μ, the mode being 39 by 30 μ, the average 41.03 by 30.85 μ. The ratio of length to width varies from 1.2 to 1.5, 1.3 occurring more than any other. Papillae are mostly apical, an occasional lateral one being found. They are prominent, the average of 25 measurements being 2.75 μ in height and 5.9 μ in width. Sporangia are mostly terminal, but frequently intercalary. Intercalary sporangia are sometimes normal in both size and shape, but many functioning sporangia have been observed, very small, spherical, and lacking the apical papillae. Such were considered out of the ordinary and were not included in spore measurements. The wall of the sporangium gives the true cellulose reaction to the chloroiodid of

zinc stain, described by Stevens (*25, p. 300*) and other authors. The figures in Plate 10, E, G, and H, are photomicrographs of typical sporangia before the escape of zoospores. These figures bring out clearly the apical papillae. Figures C and I in Plate 8 are camera-lucida drawings of typical sporangia.

Chlamydospores are sometimes very abundant in pure culture. They are spherical, thick-walled as compared with sporangia, and in some media slightly brownish in color. They, too, may be either terminal on the ends of short branches or intercalary. They are relatively large, measuring from 27 to 39 μ in diameter, 33 μ being the mode, as indicated by Figure 2, depicting the results of 200 measurements. Plate 10, F, is

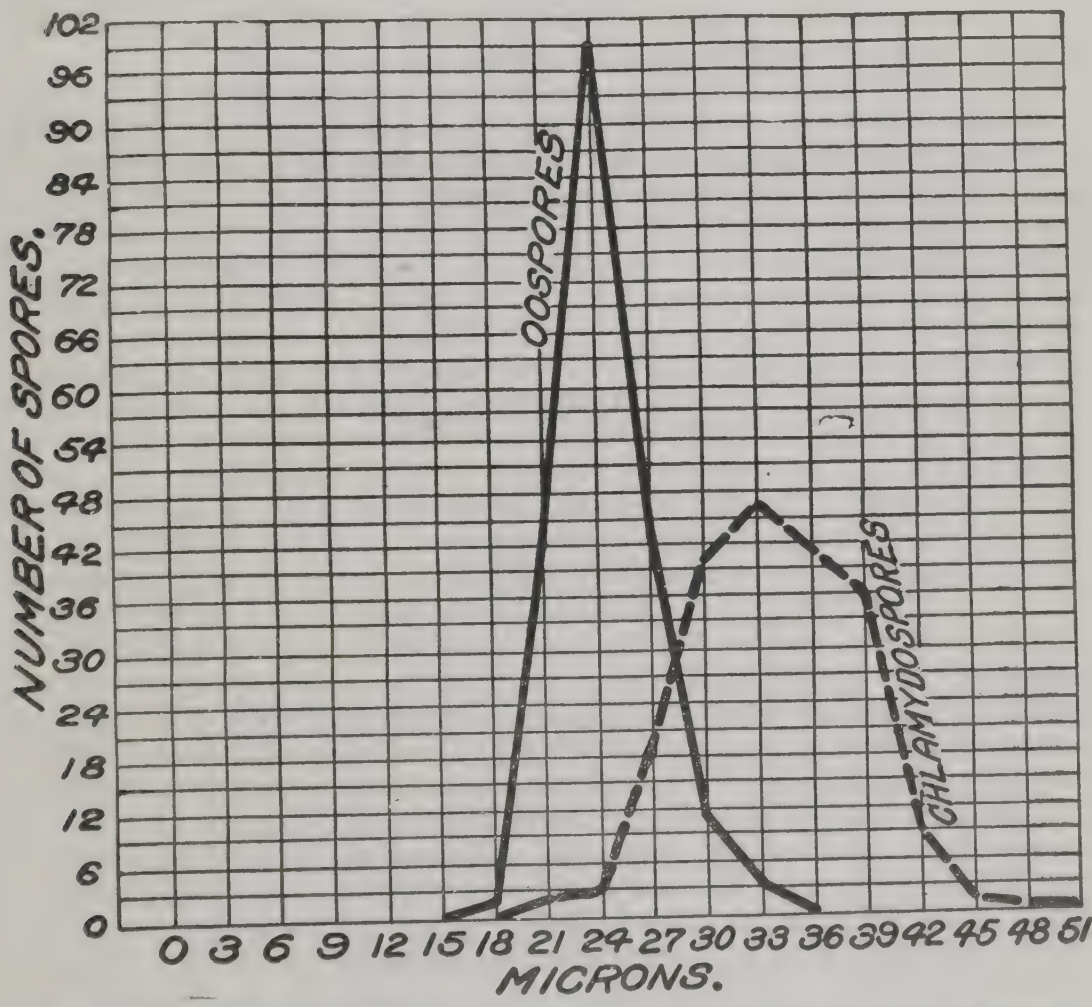

FIG. 2.—Graph showing variation in diameter of oospores and chlamydospores of *Phytophthora parasitica* var. *rhei*.

a small, laterally attached chlamydospore, and Plate 8, A, one that is intercalary.

Oospores are also found abundantly in pure culture, especially in some of the more nutrient media, such as oatmeal or lima bean agar, though they have been observed very thickly at times in corn meal agar, in the small piece of the medium with which the transfer was made from one tube to another. Oospores are spherical, ranging from 21 to 27 μ in diameter, the mode being 24 μ (fig. 2) and the mean a fraction less than 25 μ. The type of fertilization of the oogonium is always amphiginal, as described originally by Pethybridge (*18, p. 536*) and shortly after by Dastur

(*10*). Figures A to D, in Plate 10, are photomicrographs of typical specimens. Two views of the same oospore are shown here, A with the focus on the stalk of the oogonium and B on the oospore wall. Another oospore and a sporangium are also in the field, but neither is in focus. C shows especially well the relation between oogonium and antheridium, arising from separate hyphae. Plate 8 includes camera-lucida outline drawings of oospores, with the oogonium arising from the same (H) and from different (G) hyphae. Oospores are thick-walled, sometimes hyaline, sometimes distinctly brown, depending upon the substratum. The irregular thickening of the outer wall of the oogonium after the oospore is mature, mentioned by Dastur (*10, p. 202*), is often seen in oatmeal agar. The antheridia are variable, having been seen in all gradations from half the diameter of the oospore and almost square in shape to small and circular in outline. Antheridia are persistent.

Hyphae are slender, though variable, and full of granular protoplasm in younger cultures. A rapid flowing or streaming of the protoplasm has been observed under higher magnifications in young cultures on plates of clear corn meal agar. This flowing can be seen for only a few seconds after the cover of the plate is removed; the drying out of the cultures appears to stop it. The type of mycelium varies considerably with different culture media. Various features described in different papers have been observed. There are no septa in the younger hyphae, but in older cultures septations are frequent. Plate 10 shows hyphae as seen in microscopic mounts. Irregularities of the most extreme nature, even approaching the tuberculate structures mentioned by Rosenbaum (*21, p. 24*) as a characteristic of *Phytophthora syringae* Klebh., have been seen occasionally. Hyphal walls and septations consist of true cellulose.

Zoospore production in Phytophthora has been repeatedly described. The manner of their development and emergence from the sporangium is in this species apparently identical with that in *Phytophthora parasitica* Dastur as described by Dastur (*10, p. 194*). One feature that was particularly noticeable with this organism was the rapidity with which zoospore development occurs when a water mount is made from a culture of the right age and condition. When cultures are maintained at room temperature, zoospore production can be observed at any time within a few minutes. Often less than a minute from the time the mount was made sufficed to note the beginning of activity. Sometimes 15 or 20 minutes are required. Normally, activity begins in less than 5 minutes, during which time the changes in the protoplasm can be observed. This feature, together with the abundance of sporangia produced on some of the ordinary media, the long life of the organism in culture, and other characters mentioned in this paper seem, in the writer's opinion, to make it particularly desirable to use for laboratory study in plant pathology courses.

A motion-picture film has been prepared, as noted elsewhere by Godfrey and Harvey (*11*), partly for the purpose of showing to best advantage the details of the development and escape of the zoospores. Through the courtesy of the motion-picture laboratory portions of that film which show different stages in the process are reproduced in Plate 11. The first row shows the protoplasm rounded out into zoospores, with cleavage lines distinctly visible. This stage is more clearly brought out in Plate 10, H, which shows a specimen killed with osmic acid fumes just previous to the escape of the zoospores, and treated with chloroiodid of zinc (*25, p. 300*). This stains the sporangium wall a light blue or purple and the

contents yellow. Next occurs a sudden bursting outward of the papilla, which can be seen in strip B. It was distinctly seen that there is no bursting of a membrane, but rather a stretching of the apparently gelatinous substance of the papilla itself into a thin plasma membrane, which ordinarily remains intact, holding the contents together, until a large part or rarely all of the protoplasm is out of the sporangium. The membrane then gives way all at once, possibly by becoming dissolved rather than bursting at any one point, and the zoospores free themselves from one another and swim away in all directions. This stage is shown in Plate 11, C. By comparing the extremities of the strip, it will be seen that change is rapid at this stage. The pictures are taken at the rate of 16 per second. At this stage also flagellae may often be seen lashing out on all sides of the protoplasmic mass at the mouth of the sporangium. With a large sporangium, which may contain as many as 64 zoospores, those that escape first in a single body usually become free and swim away before the last are out. This condition is evident in E. In such a case it is interesting to watch the amoeboid movements of the final parts of the protoplasmic mass. Figures F and G show the final stages of this very interesting phenomenon. Plate 8, B, also shows different stages of zoospore emergence, as drawn with the aid of the camera lucida.

Zoospores are so active when first they become free that it is impossible to catch any but the faintest glimpse of the flagellae. Many observations under different conditions, however, revealed bit by bit the various phases of their activity. Perhaps the best view of them in their normal activity was obtained in the slightly dull light of late afternoon, with the direct light from the blue sky and a 2.5-mm. Zeiss water immersion lens. This particular light appeared to provide the correct conditions for displaying the delicate flagellae. These observations were possibly still further favored by a low temperature, which appears to slow up the movements of the organism.

The zoospores are the usual Phytophthora type, kidney-shaped, biciliate, contents granular, and nucleus indistinctly visible in the unstained specimens. They measure 8 to 9 by 11 to 13 μ while active, gradually rounding up as they lose their activity. Eventually they are globose and measure from 8 to 10 μ in diameter. The flagellae are attached close together in a longitudinal groove on one side. As the swarm spore swims along with its more pointed end forward, the anterior flagellum is the shorter, being approximately twice the length of the spore. It lashes violently back and forth. The posterior one is about 2½ times the length of the spore and drags along without much motion. As the zoospore gradually comes to rest, slight enlargements may be seen on the ends of the flagellae. As these droplets become larger the flagellum shortens by what appears to be a process of deliquescence. This has been observed in connection with some of the water molds and has evidently been observed in Phytophthora as well, for Sawada pictures it in connection with *Phytophthora allii* Sawada (*22*, *Pl. 1*). The droplets approach the spore as it becomes quiet and round, and it is presumed that they drop off, though this has not been confirmed by the writer. One spore under observation for 15 minutes showed the droplets still not quite in contact with the spore, and it was lost before any further change took place. Most zoospores after having come to rest do not have these droplets attached. Plate 9, A to C, are camera-lucida drawings made from fixed materials. A slide showing swarm spores in various stages of activity was held for about 10 seconds over a bottle of

2 per cent osmic acid. This killed the spores instantly. Then very dilute aqueous gentian violet solution was applied, resulting in the immediate staining of the spores. The flagellae too, being at rest, were distinctly visible, and in certain cases their position would seem to indicate their instantaneous cessation of normal active motion. The osmic acid darkened the oil contents of the spores sufficiently to interfere with the view of the structure of the protoplasm; consequently no attempt was made with this material to draw the cell contents. Figure A of Plate 9 shows spores in varying positions, with flagellae. Figure B shows different stages in their deliquescence and disappearance. Here is seen a rounded spore that has come to rest with the droplets still attached. Figure C shows zoospores that are abnormal or that had not sufficient time, before being killed, to round out into normal.

Within an hour or two after motility ceases zoospores often begin to germinate. Plate 9, D, consists of drawings of germinating zoospores in different stages of growth. Germination in water sometimes results in the formation of small conidia, normal in appearance, as shown in F. These may produce zoopores, or may germinate by germ tubes and continue the vegetative growth. At times also small chlamydospores may be formed (Pl. 9, E). Germination on a plate of corn meal agar resulted in the development of normal vegetative hyphae such as occur when the host plant is infected with zoospores. Figures K, L, and M in Plate 10 consist of photomicrographs of germinating zoospores in different stages.

CULTURAL CHARACTERISTICS

The appearance of this Phytophthora, as of others, differs considerably on different media. It was not grown on an extensive list of media for the express purpose of making comparisons, but on those used for special purposes, observations were made as follows:

Corn meal agar (30 gm. corn meal, 20 gm. agar, 1,000 cc. water). At first growing on and beneath the surface, the organism very quickly (usually by the second day) produces aerial hyphae which, in mass, present a loose, fluffy, semitransparent appearance rather than a pure white, cottony appearance, such as occurs with many fungi producing colorless hyphae. Mature sporangia may be found on the fourth day, though they are not present in greatest abundance until the culture is from 10 days to 2 weeks old. Chlamydospores and oospores may often be found attached to the glass above the surface of the medium, especially if a thin layer of the agar were left there while the tube was being slanted.

Oatmeal agar (100 gm. ground Quaker oats, 20 gm. agar, 1,000 cc. water). The growth is similar to that on corn meal agar, except that the aerial mycelia become more dense. This medium is very good for the development of oospores, which occur profusely in the surface layer after it dries out a little.

Lima bean agar (100 gm. lima beans in 2 per cent agar). Growth is similar to that on corn meal agar, except that the surface and subsurface development is more pronounced and of longer duration before the aerial hyphae develop, and the latter growth is not nearly so dense. This medium is a very good indicator as to the purity of a culture. Bacterial contaminations become evident very soon, if they are present. It is also good for the production of oospores, which are sometimes found in great abundance embedded in the surface layers.

Various sterilized vegetable plugs were used. Dastur has noted (*10, p. 217*) that *Phytophthora parasitica* failed to develop on sterilized carrot and sterilized sweet potato. The writer's strain grew very readily on these media, producing a tough superficial mat of hyphae at first, and after a few days a dense white cottony growth. The growth was similar on string bean plugs and on sterilized potato. On none of these vegetable plugs were sporangia produced to any great extent.

The organism is very tolerant to acid, at least to that found in the rhubarb stem, as might be expected from its habits in nature. It grew in pure culture, though somewhat slowly, in rhubarb decoction that titrated roughly to phenolphthalein 0.07 N (+70 Fuller's scale). Haas (*12*) gives the following figures for the acidity of different parts of the rhubarb leaf stalk; basal part, actual acidity or concentration of free hydrogen ions, determined electrometrically, 0.0007 N; intermediate part, actual acidity 0.0005 N, total acidity 0.1578 N; green part below leaf blade, actual acidity 0.00022 N, total acidity 0.1681 N. As Haas states, there is here shown a great difference between the total and actual acidities, which difference is probably advantageous to the plant's metabolism in that it helps to maintain fairly constantly that actual acidity which is most favorable. Even the actual acidity is unusually high, however, as is shown by the same author in another paper (*13*), in which he compares it with soybean, the former having a P_H of 3.36 (acidity 0.00044 N) and the latter of 5.85 (acidity 0.0000014 N). The acidity, however, does not prevent the active invasion of the Phytophthora into all parts of the stalk, as has been shown repeatedly by observations in the field and inoculations into rhubarb stalks in the laboratory. Neither is high acidity requisite to the rapid growth of the organism, as is shown by its ability to grow on a wide variety of artificial culture media and on many common fruits and vegetable roots.

In the winter of 1917 inoculations were made into the starchy food-storing roots and tubers of various plants. This was repeated in 1920–21 with a wider range of hosts, including certain fruits. The roots and fruits were first thoroughly surface sterilized by immersing for 10 to 15 minutes in 1 to 240 formaldehyde, then wrapped in a dry towel and allowed to stand overnight. The inoculations were made by cutting a deep slit with a sterile scalpel and inserting the inoculum from a vigorous culture about 8 days old. The specimens were then placed in moist chambers and allowed to stand 2 to 4 days.

Stayman Winesap and Grimes Golden apples were both rapidly attacked, the fungus growing in toward the center as rapidly as it spread beneath the skin. The decay of the fruit was light brown or tan in color and of a soft and mealy consistency. Typical hypae could be found at any point in the advancing edge of the decayed area. Reisolations were easily secured free from contaminations, from the advancing edge beneath the skin, or from the interior of the fruit. Seeds that had been reached by the fungus in one of the fruits when planted in a plate of corn meal agar gave a pure culture of it. Parsnips (*Pastinaca sativa* L.) were readily attacked with a soft, wet, but not watery decay and a characteristic pinkish or light purplish discoloration of the surface. Carrots (*Daucus carota* L.) also were rapidly decayed, with a clear watery condition developing darker in color than the normal. The decay spread to the growing leaves and quickly involved them. Turnips (*Brassica rapa* L.) decayed more slowly and did not lose their hard texture, but a distinct blackening of the interior occurred. Sweet potatoes (*Ipomoea*

batatas Poir) were readily attacked, with a firm dryrot, and the production of a distinct rose-geranium odor that is always evident in soft-rot due to *Rhizopus nigricans* Ehr. (*14, p. 341*). White potato (*Solanum tuberosum* L.) decayed rapidly with exactly the same symptoms as those described by Pethybridge for *Phytophthora erythroseptica* Pethyb. (*18*). The pink color, though not evident when the tuber was first cut, developed in the course of half an hour's exposure to the air. This was especially striking when first seen, because of its unexpectedness. It was followed by a change to dark brown as with the other species. Tomato fruits (*Lycopersicon esculentum* Mill), green and ripe, were rapidly attacked, with the spread of the fungus through the interior with brown discolorations. No aerial mycelium developed. Dasheen (*Colocasia antiquorum* Schott) corms were not attacked by the fungus, though the same moisture and temperature conditions prevailed as with all the others. Onion (*Allium cepa* L.) was not decayed, except slightly in the particular layer in which the inoculum was placed.

The whole series was in duplicate, with a third specimen punctured, but not inoculated, as a control. All controls remained intact. Later the entire series was repeated with identical results. Reisolations of the organism into pure culture were made from all except the onion and the dasheen. Plate 12 consists of photographs of the different specimens, showing the nature of the decay produced.

Temperature has a marked influence on the development of the organism. Corn meal agar plates were placed in a series of Altmann controlled temperature incubators ranging from about 4° to 40° C., with intervals of from 2° to 3°. The cardinal temperatures, as indicated by extent of vegetative growth, were approximately as follows: minimum, 13°; optimum, 30°; maximum, 36°. It will be seen that this organism is like the tropical forms in having very high temperature requirements. This explains the facts noted elsewhere in regard to season of active parasitism.

INOCULATIONS INTO OTHER PLANTS

No very wide range of growing plants was inoculated, due partly to lack of time and partly to the unsatisfactory results of previous investigators. In this connection Wilson (*30, p. 77*) says:

> A comparison of the results published by the various authors tends to throw decided doubt upon the value of this method [cross inoculations] of delimiting species in this genus, as practically any species of Spermatophyta, which is in nature subject to the attacks of any Phytophthora, is likely under laboratory conditions to be more or less severely attacked by almost any other species. Indeed, some of the hosts recorded for various species of the genus are not known to harbor these fungi in nature. It would appear, then, that the parasitism of Phytopthora is of such a low order that it will not admit of their being differentiated into races as are certain of the Uredineae for example.

To consider one case: *Phytophthora cactorum* (Lebert and Cohn) Schrot, originally isolated from a species of cactus, was later found in Japan and America (*20*) on ginseng and still later in Europe (*31*) and America (*1, 28*) on apple and pear. Such inoculations may be, however, of direct value in some cases, and a few were made with the rhubarb Phytophthora on hosts of closely related species to determine whether the signs, if any, produced on the plant would be the same as those caused by the fungus originally obtained from it.

Castor bean (*Ricinus communis* L.) inflorescences were cut from plants and inoculated in the laboratory, as was done by Dastur with *Phytophthora parasitica*. Male and female buds were attacked and discolored,

PLATE 6

A.—Seedling rhubarb in pots, the one inoculated and the other not.

B.—Rhubarb plant in the experimental field inoculated July 20, 1920, by applying the fungus from pure cultures at the wound left by pulling leaf stalks. Photograph taken six days after the inoculation. The plant was killed.

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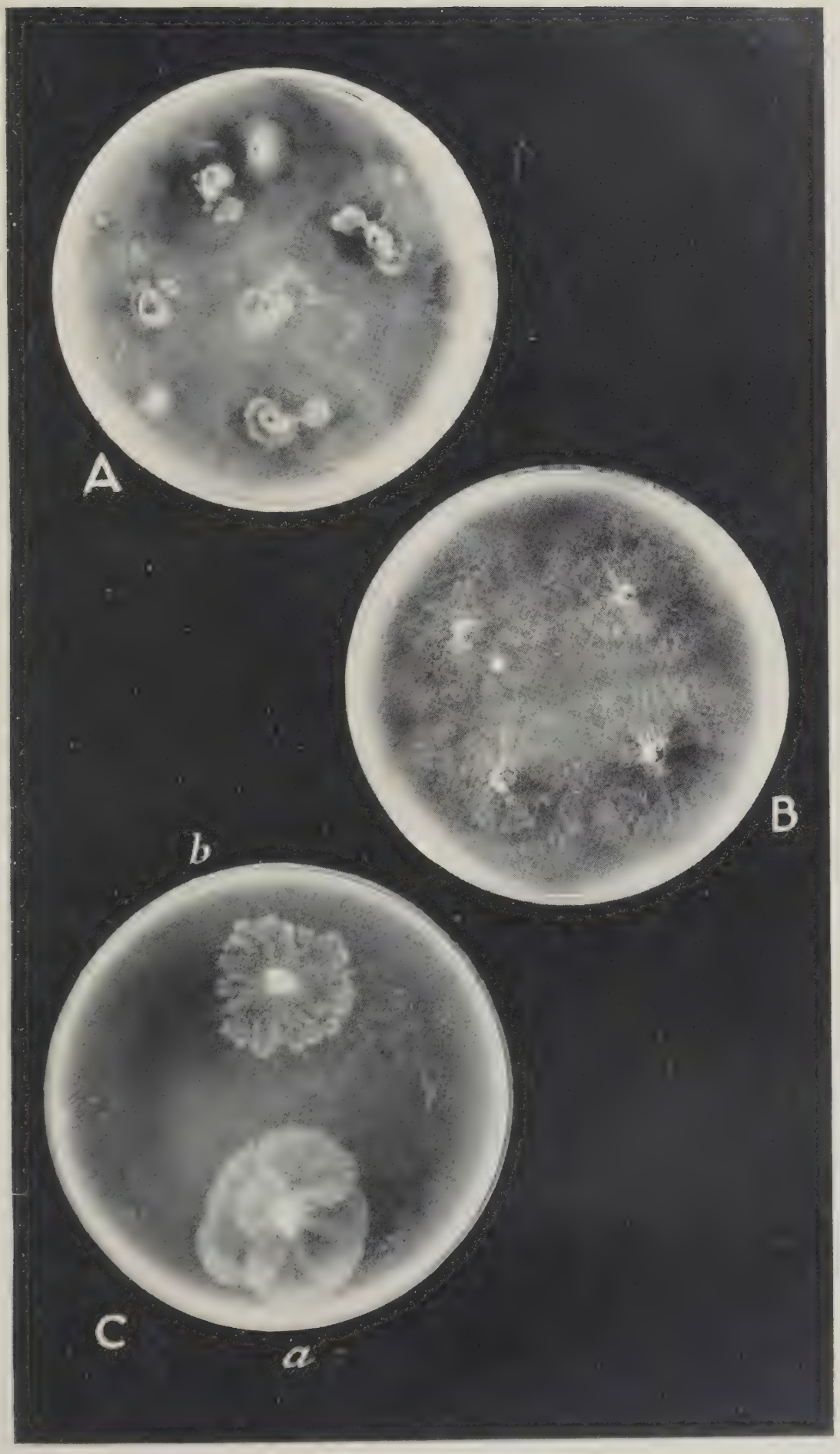
PLATE 7

Petri dish cultures of *Phytophthora parasitica* var. *rhei*.

A.—A contaminated culture.

B.—The same strain secured in pure culture by inoculating into an apple and reisolating from the advancing edge of decay.

C.—*Phytophthora parasitica* var. *rhei* (a) and *Phytophthora terrestris* Sherb. (b) compared, on corn meal agar.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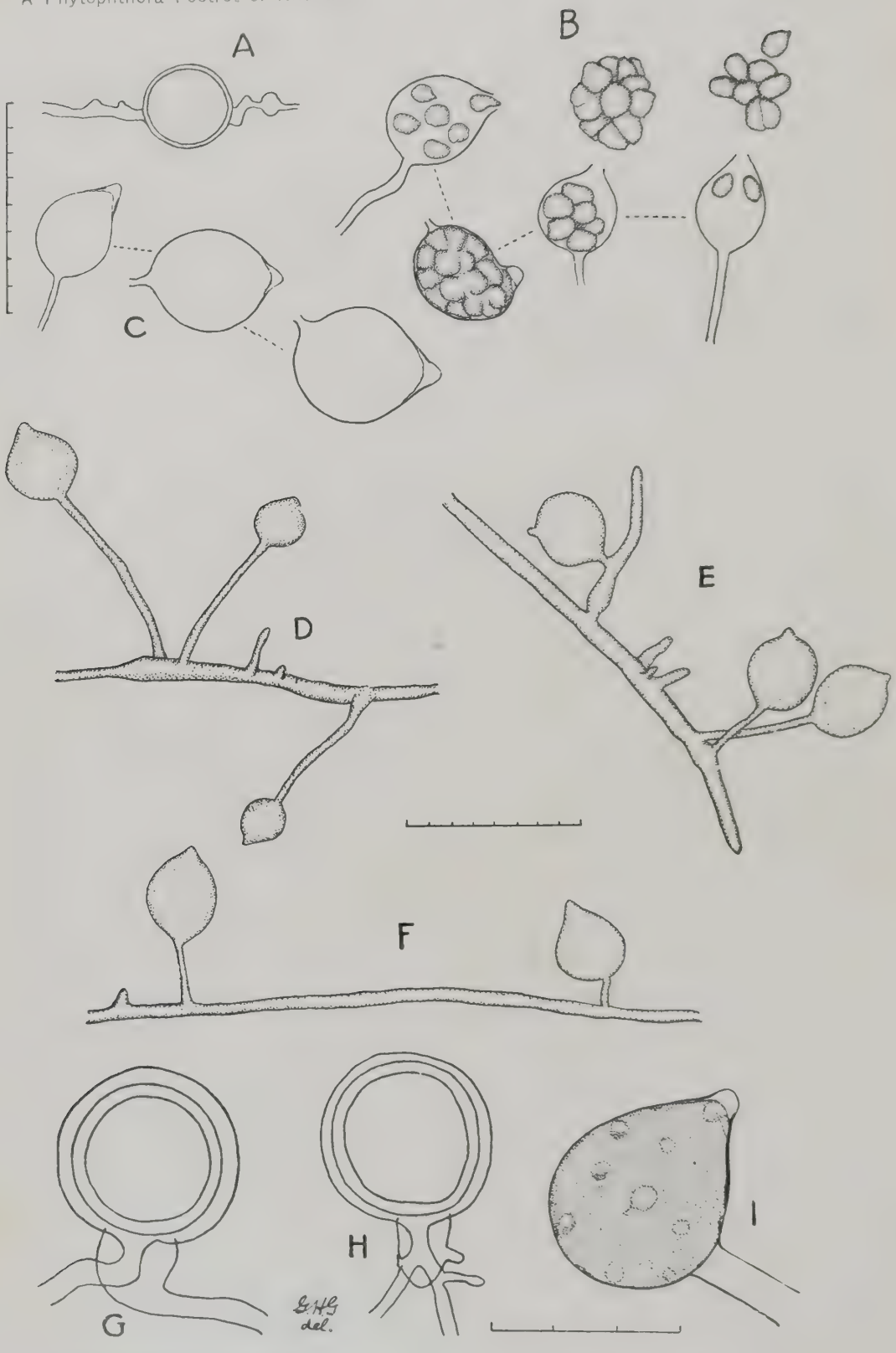
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PLATE 8

Camera-lucida drawings of *Phytophthora parasitica* var. *rhei*.

A.—Outline drawing of an intercalary chlamydospore, showing the thick wall, somewhat exaggerated.

B.—Various stages in zoospore emergence.

C.—Sporangia in outline, showing relation of granular protoplasmic contents to the papillae.

D, E, and F.—Sporangia as found in pure culture, attached to irregular hyphae.

G.—Oospore, showing relation of oogonium and antheridium on separate hyphae.

H.—Oospore with the antheridium apparently an outgrowth of the same hypha as that from which the oogonium developed.

I.—A sporangium drawn to show better than in C the relation of protoplasm to the papilla. Note also the thin wall of the papilla in contrast with the thick sporangium wall ending abruptly.

Each scale division represents 1/100 mm.

PLATE 9

Camera-lucida drawings of *Phytophthora parasitica* var. *rhei*.

A.—Different views of zoospores killed with osmic acid fumes and stained with dilute gentian violet.

B.—Zoospores gradually coming to rest, showing enlargements at tips and disappearance of the flagellae.

C.—Irregular zoospores, one of them with four flagellae, consisting probably of two individuals not yet pulled apart. The others also were killed shortly after emergence, before they had time to attain normal shape.

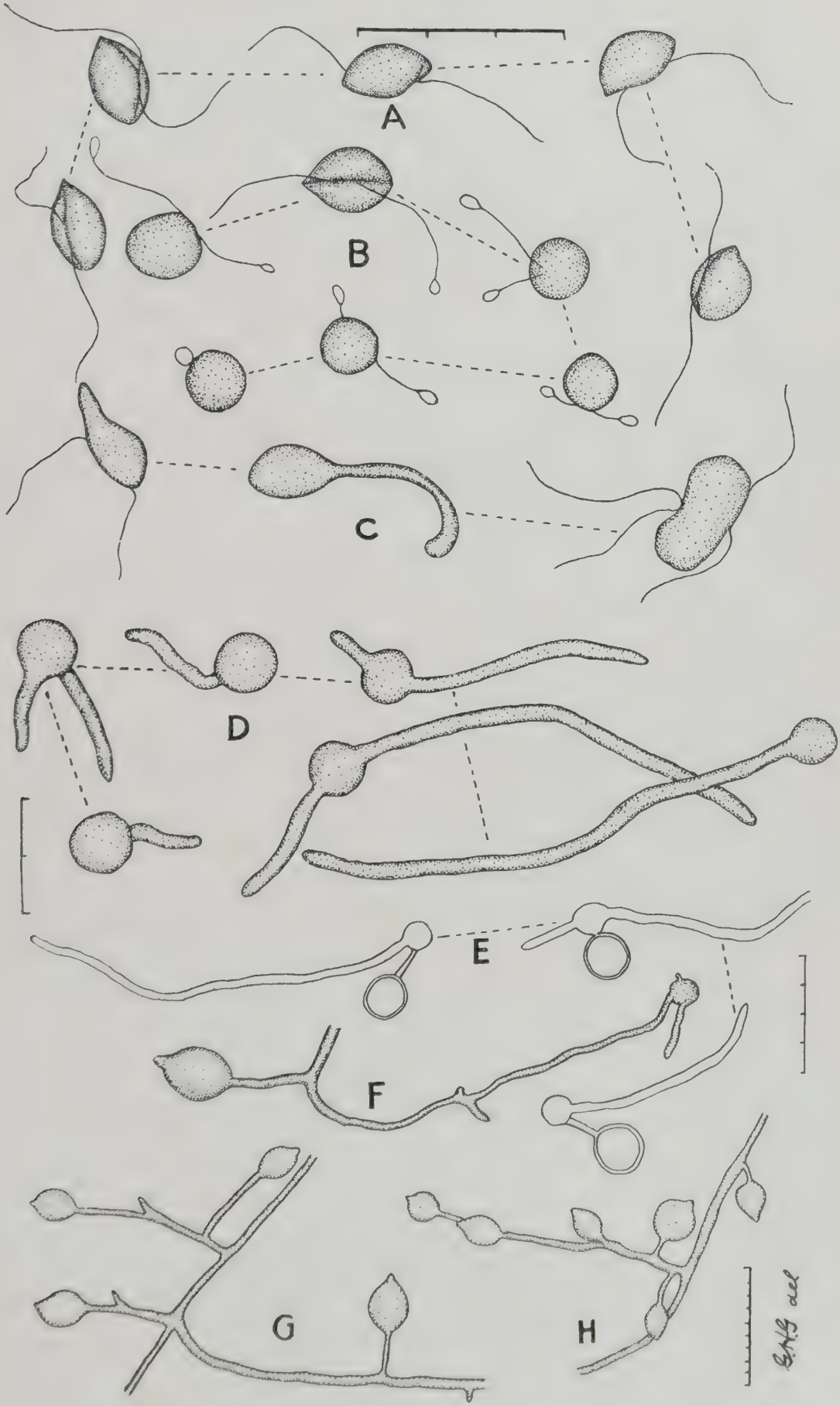
D.—Germinating zoospores in different stages.

E.—Germinated zoospores producing chlamydospores.

F.—Germinated zoospore with production of sporangium.

G, H.—Sporangium production from pure cultures.

Each scale division represents 1/100 mm.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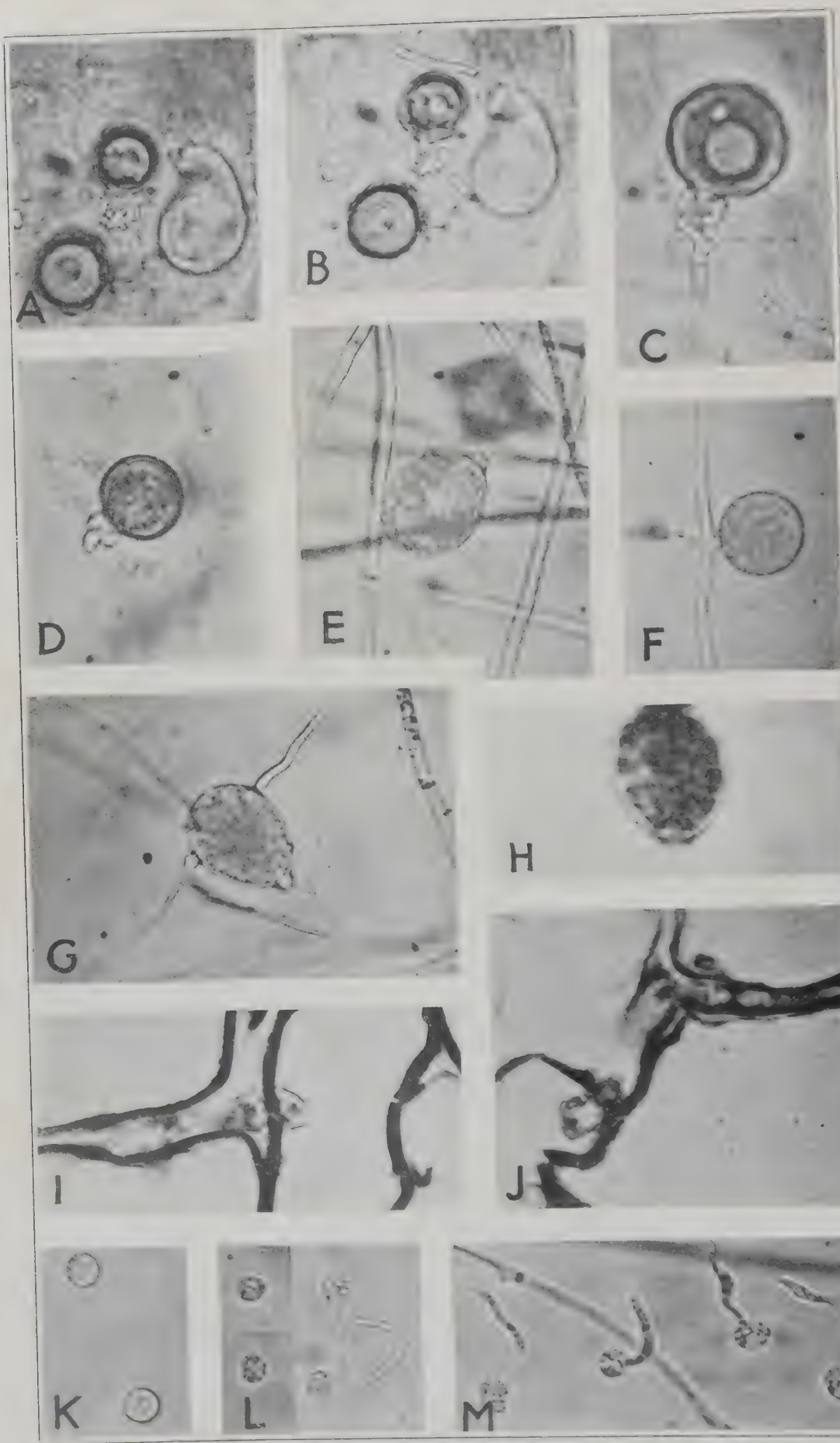
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PLATE 10

Photomicrographs of *Phytophthora parasitica* var. *rhei*.

A.—(Center) oospore, with focus on stalks of antheridium and oogonium.

B.—Same with focus on oospore wall.

C.—Another oospore, showing attachment of the oogonium and its relation to the antheridium.

D.—Another oospore.

E.—A sporangium, showing granular protoplasm and papilla.

F.—A laterally attached chlamydospore.

G.—An intercalary sporangium.

H.—A sporangium killed with osmic acid fumes just before emergence of the zoospores would have taken place. After killing the slide was treated with chloroiodid of zinc, which stained the walls a light blue and the protoplasm yellow. Note the cleavage at this stage into distinct individuals. The papilla also is clearly shown.

I, J.—Intercellular hyphae and cell wall penetration. Note in both the slender hypha at point of penetration and enlargement afterward. J shows what appears to be a typical haustorium.

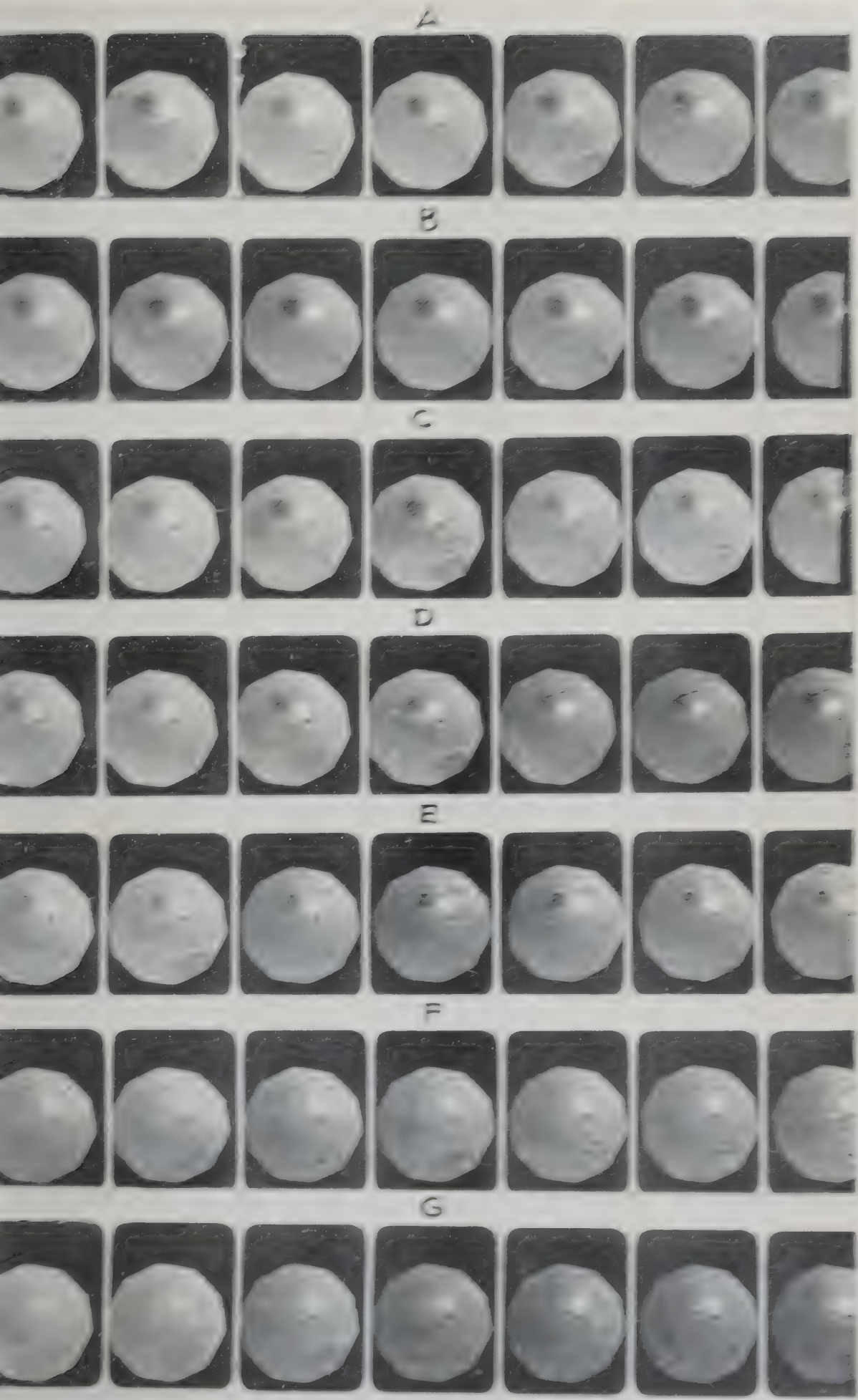
K, L, M.—Various stages in zoospore germination.

PLATE 11

Progressive stages in the emergence of zoospores of *Phytophthora parasitica* var. *rhei*, as shown by reproductions from a motion-picture film.

Strip B follows A immediately on the film, and shows the rapid bursting outward of the papilla. The spot just in front of the sporangium is a zoospore from another sporangium. It has come to rest in a very unsatisfactory position. The glow of light just at the opening of the sporangium is another unsatisfactory feature which could probably be eliminated with more experience.

Strips C to G were taken from widely separated intervals in the film, and show subsequent stages in the escape of zoospores.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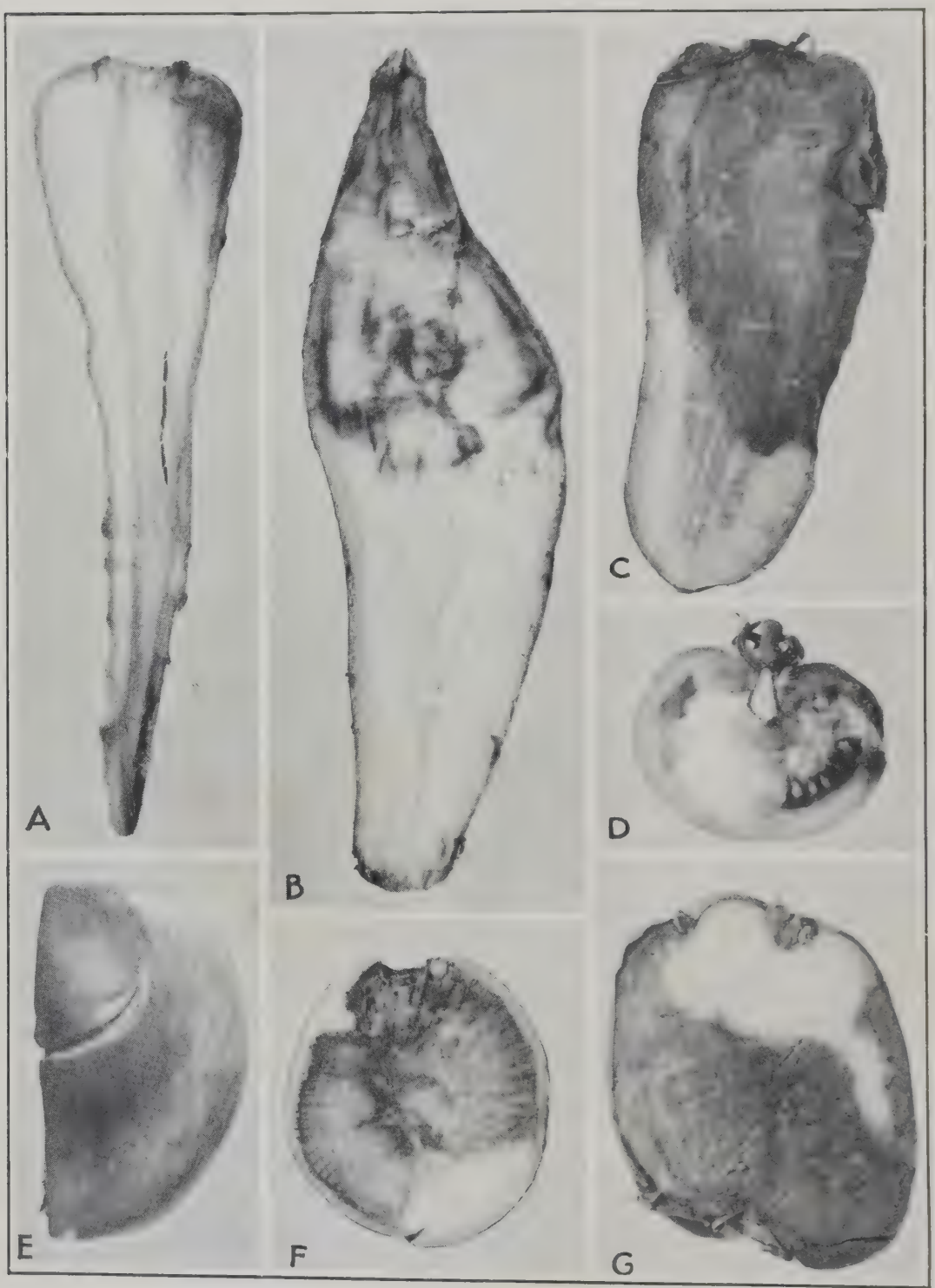
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PLATE 12

Effect of *Phytophthora parasitica* var. *rhei*, on different plants, the darker area in each case being the region penetrated by the hyphae after three days' incubation.

A.—Parsnip.
B.—Sweet potato.
C.—Carrot.
D.—Tomato.
E.—Stayman Winesap apple.
F.—Turnip.
G.—Potato.

SAND DROWN, A CHLOROSIS OF TOBACCO DUE TO MAGNESIUM DEFICIENCY, AND THE RELATION OF SULPHATES AND CHLORIDS OF POTASSIUM TO THE DISEASE[1]

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DESCRIPTION OF SAND DROWN AND ITS EFFECTS

The disease of tobacco which has received the name "sand drown" bears no essential relation to true drowning from excessive moisture in the soil. On the contrary, as indicated in the name itself, the disease is likely to be most severe on deep, sandy soils which never become water-logged. The trouble is aggravated by heavy rainfall, as suggested by the word "drown;" but, as will be subsequently developed, the effects of the rainfall are chiefly those of leaching rather than of the presence of the water itself. The dominant symptom is a characteristic type of chlorosis in which the yellow as well as the green pigments of chlorophyll are affected. Thus, in the affected tissue the color is a dull, very pale yellow, and in extreme cases almost pure white. This color is readily distinguished from the clear lemon or orange color commonly observed in the healthy leaf which has been exposed to darkness for a brief period of time or has undergone partial curing. The chlorosis begins on the lowermost leaves and at the tips of the leaves affected. In advanced stages practically the whole area of the leaf is involved and nearly all the leaves of the plant may be affected. The advance of the chlorosis is from the tip and the margin toward the base and the central zone of the leaf. Successive stages in the progress of the bleaching effect in the leaf are shown in Plates 2 and 3. Sand drown rarely appears in the field till the plants have attained considerable size, more commonly after topping, so that the leaves usually attain the normal size and shape, as is indicated in Plate 1. The veins of the leaf, as well as the tissue immediately adjoining the veins, show a tendency to retain the normal green color long after the remaining leaf tissue has become blanched.

The disease is scarcely less evident in the cured leaves than in the green leaves in the field. In the flue-cured district normal leaves when properly cured have a bright lemon to orange color, and in other districts the colors range through various shades of brown with either a

[1] Accepted for publication Sept. 1, 1921. Cooperative investigations of the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the North Carolina Department of Agriculture.

reddish or a greenish cast. On the other hand, the leaves or the por tions of leaves affected with sand drown show after curing a dull, fade appearance similar to that observed in the diseased leaves in the fiel Since color is of great importance in determining the commercial valu of most types of tobacco, it is easily seen that sand drown ma greatly lower the market price of the affected leaves. This appli with special force to the sale of cigar-wrapper leaf. Moreover, san drown greatly reduces the weight of the cured product; in fact th affected parts of the leaf are remarkably thin after having been cure For example, in a test with flue-cured tobacco the normal leaf wa found to be 45 per cent thicker and 65 per cent heavier per unit are than the diseased leaf. As would be expected, the tissue of disease leaves is dry and lifeless. Thus, sand drown affects unfavorably th color, weight, body, and elasticity of tobacco leaf.

OCCURRENCE OF SAND DROWN

The disease first came under observation about 1912 at the Tobac Station of the North Carolina Department of Agriculture located Oxford, in connection with the cooperative investigations in progress that point. The same trouble also was seen on some of the farms tobacco growers in the vicinity. It has appeared each year on t tobacco grown at the Oxford station, but it soon became apparent th weather conditions and the kinds and quantities of fertilizers appli affect in a marked degree the appearance and course of the disease. general, it may be said that with approximately normal rainfall and t use of fertilizers of the grades and the quantities that have been mo widely employed in tobacco culture sand drown is not commonly se in sufficiently severe form to attract attention or to cause serious lo On the other hand, the malady is likely to be widespread in seasons heavy rainfall in those sections in which the dominant soil types distinctly sandy in character. For example, this type of chlorosis frequently seen on the more sandy tobacco soils of the Connecti Valley in excessively wet seasons. As will be pointed out, the use certain fertilizer salts will lead to symptoms of sand drown even in s sons of normal rainfall and on the heavier types of soil, although remains to be determined just how generally this action may be obser under varied soil and weather conditions. It is possible, also, t material injury to the color and other properties of the cured leaf m result from mild forms of the trouble which would not be clearly rec nizable in the field. In pot cultures sand drown can be easily indu and cured at will.

RELATION OF SULPHATES AND CHLORIDS TO SAND DROWN

As a feature of the cooperative investigations at the Oxford (N. Tobacco Station, fertilizer plot tests have been in progress for sev years in which various forms and rates of application of nitrogen, ph phoric acid, and potash are compared. The soil is classed as Durh sandy loam and is not fertile. Sand drown has frequently appeared the test plots. On most of the plots the tobacco has been fertilized w sulphate of potash in combination with other materials, and it observed that increase in the rate of application of the sulphate cle increased the severity of sand drown in the tobacco. On plots recei

muriate of potash in place of the sulphate, other conditions being the same, there was a marked decrease in the amount of disease and an appreciable increase in the size attained by the plants. In a second set of plot tests, in which a more detailed comparison between the muriate and sulphate of potash was made, the striking effect of the sulphate in inducing or aggravating the disease was fully confirmed. In the first set of plots the standard rate of application of the potash salts was 80 pounds per acre actual potash (K_2O), while certain plots received 40 and 160 pounds per acre. In the second series of plots the rates of application were 12, 24, 36, and 80 pounds per acre. In the first series the normal application of phosphoric acid was 64 pounds per acre in the form of 16 per cent acid phosphate, and the normal application of nitrogen was 32 pounds, various forms being used. Even the smallest applications of potassium sulphate produced marked effects in increasing sand drown. It is obvious that these quantities are much too small to produce injury by unduly increasing the concentration of the soil solution, and, moreover, corresponding quantities of potassium chlorid have shown no injurious action of any sort. The peculiar action of the sulphate, contrasting so sharply with the chlorid, seemed perplexing, for, while in recent years considerable stress has been placed on the necessity of an adequate supply of sulphur for the plant, no case has been previously recorded, so far as known, in which small quantities of sulphates caused serious injury under field conditions.

A series of single-row tests was carried out at the Oxford station in which sodium chlorid, sodium carbonate, and calcium carbonate were used, in addition to the normal application of potassium sulphate, dried blood, and phosphorous in the form of so-called precipitated bone, a finely divided dicalcic phosphate. The common salt and sodium carbonate were used at the rate of 200 pounds and the calcium carbonate at the rate of 1,000 pounds per acre. The sodium carbonate was not effective in preventing sand drown, and the calcium carbonate was only partially effective, neither of these materials causing appreciable increase in growth of the tobacco. On the other hand, the sodium chlorid (ordinary commercial "salt," sold in bulk) probably increased the growth of the tobacco by fully 100 per cent, and there was no sand drown. The next season another series of row tests was carried out in which chemically equivalent quantities of the chlorids and sulphates of sodium, calcium, and magnesium were used, in addition to a standard application of 100 pounds per acre each of ammonium nitrate and monopotassium phosphate. All salts were approximately chemically pure, and the quantities used were on the basis of 100 pounds sulphate (SO_4) per acre or the equivalent quantity of chlorin. In this case the sodium chlorid and the calcium chlorid were without effect in controlling the sand drown. Both the sulphate and the chlorid of magnesium, however, showed a marked effect in preventing the disease; and this observation first gave the clue to the situation, as will be shown in subsequent paragraphs.

RELATION OF ORGANIC MATTER TO SAND DROWN

On the fertilizer test plots at Oxford several different sources of nitrogen were used—namely, nitrate of soda, ammonium sulphate, dried blood, and cottonseed meal. These materials were applied at rates to furnish about 32 pounds nitrogen per acre, the quantity of cottonseed

meal employed being 500 pounds. It was quickly seen that the cottonseed meal showed a decided tendency to reduce or to delay the symptoms of sand drown while ammonium sulphate accentuated the trouble. There was no notable difference between the action of nitrate of soda and that of dried blood. Once more the injurious action of sulphates is in evidence, but, in addition, it is clear that only 500 pounds per acre of organic matter in the form of cottonseed meal exercises some preventive action against the disease. Apparently there is some similarity in the action of certain chlorids and that of cottonseed meal calling for explanation. Different rates of application of nitrogen in the form of dried blood produced no notable effect on the disease, so that neither the form nor the quantity of nitrogen in the fertilizer can be considered as being of primary importance.

Another series of plot tests was conducted for several years at Oxford dealing with the fertilizing value of tobacco stalks and stems (leaf midrib) and barn manure for tobacco. For purposes of comparison the normal mixture of sulphate of potash, dried blood, and acid phosphate also was included in this series. The tobacco stems were applied at rates ranging from 1,000 to 2,600 pounds per acre, supplemented with sufficient quantities of dried blood and acid phosphate to furnish the normal application of 32 pounds nitrogen and 64 pounds phosphoric acid per acre. The tobacco stalks were applied at the rate of 2,700 pounds per acre, also supplemented with acid phosphate. The manure was used alone, in combination with sufficient acid phosphate and sulphate of potash to furnish the normal formula, and in combination with hardwood ashes as a source of potash. The rate of application of the manure was 8,000 pounds per acre. As regards sand drown, the outstanding feature of these tests was that there were no symptoms of the disease except on the plots receiving only the mixture of dried blood, acid phosphate, and sulphate of potash. Here again is distinct evidence that vegetable forms of organic matter tend to prevent the development of sand drown.

BIOCHEMICAL STUDIES OF SAND DROWN

Because of the marked difference between the chlorid and the sulphate of potassium in their action on the tobacco plants, especially with regard to sand drown, experiments were undertaken for the purpose of comparing the effects of the two salts on the internal processes of the plant. A series of observations was made on the relative osmotic concentration of the expressed, filtered saps from the frozen tissues of the leaf after the midrib had been removed, the material for study being taken from the two sets of fertilizer test plots at Oxford previously referred to. The samples were taken at intervals of two to four days, beginning shortly after the plants had been topped, that is, when the plants had reached the normal stage for flowering. A single leaf near the middle of the plant was collected from a representative plant of each plot, and the same plants were used as sources of material throughout the tests. The leaves were collected in each case about 9 o'clock in the morning. In all cases the tobacco plants on the control plots receiving no potash showed distinct symptoms of severe potash hunger. The data obtained are summarized in Table I. The osmotic concentrations, in atmospheres, were calculated in the usual way from the observed lowering of the freezing point.

TABLE I.—*Osmotic concentration of the sap of tobacco leaves and the ash of the sap, as affected by the chlorid and the sulphate of potassium*

Fertilizer treatment.[a]	Osmotic concentration of sap (in atmospheres).					Osmotic concentration of ash of sap (in atmospheres).	
	July 22.	July 24.	July 26.	July 30.	Average.	July 22.	July 24.
General fertilizer test plots:							
N+P	9.346	8.396	9.093	8.913	8.937	2.929	1.869
N+P+80 pounds K_2O, as sulphate	.....	9.021	9.057	7.878	8.652	.....	6.012
N+P+160 pounds K_2O, as sulphate	11.060	10.530	9.418	9.875	10.220	6.855	7.349
P+80 pounds K_2O, as sulphate	8.937	8.949	8.576	7.987	8.612	5.940	6.266
N+80 pounds K_2O, as sulphate	8.540	8.492	8.215	7.951	8.299	5.013	4.917
Average moisture percentage of soil of plots	7.0	5.5	7.25	9.0			

Fertilizer treatment.[a]	Osmotic concentration of sap (in atmospheres).					Osmotic concentration of ash of sap (in atmospheres).	
	July 20.	July 23.	July 25.	July 29.	Average.	July 20.	July 23.
Special potash test plots:							
N+P	7.734	8.191	8.504	8.071	8.125	0.917	1.748
N+P+24 pounds K_2O, as chloride	9.574	8.720	10.240	8.600	9.283	3.086	2.868
N+P+24 pounds K_2O, as sulphate	8.768	8.780	9.298	8.167	8.753	2.712	3.351
N+P+80 pounds K_2O, as chlorid	10.070	10.670	10.760	9.959	10.360	7.710	8.191
N+P+80 pounds K_2O, as sulphate	9.635	8.805	10.280	8.528	9.312	5.182	4.929
Average moisture percentage of soil of plots	8.0	5.5	9.0	9.0			

[a] N=32 pounds nitrogen per acre, in form of dried blood. P=64 pounds phosphoric acid per acre, in form of acid phosphate in first series and precipitated bone in second series.

Concerning weather conditions during the period of observation it may be stated that cloudy weather, with showers, prevailed July 17, 19, 24, 27, and 31, with sunshine on all other days, except for a few thunder showers of brief duration. Rainfall of consequence occurred only as follows: July 17, .33 inches; July 24, 1.55 inches; July 27, 1.37 inches; July 30, 0.82 inch. The soil, which is a light, sandy loam, has a low water-holding capacity, and there was a wide range in degree of saturation during the period of study. The osmotic concentration of the sap of the plant, however, does not appear to be definitely correlated with the water content of the soil. In general, increased application of potash salts to the soil increased the osmotic concentration of the sap and the chlorid was decidedly more effective than the sulphate in this respect.

The mineral component of the osmotically active material of the sap was determined by evaporating the sap, igniting the residue, and restoring the original volume with distilled water. The data in Table I clearly show that where no potash was applied to the soil much the greater proportion of the osmotic concentration represents soluble organic material, probably carbohydrate, whereas with increasing quantities of potash salts applied to the soil this relationship is reversed, the mineral component becoming the more important. The chlorid, moreover, contributes more to the osmotic concentration of the sap than does the sulphate. These observations seem to be in line with the view which has long been held that lack of an adequate supply of potash tends to cause congestion or accumulation of carbohydrate in the tissues of the leaf.

The saps (in 10 cc. portions) from the general fertilizer test plots collected on July 24 and those from the special potash plots collected on July 23 were employed in studying the quantity and character of the ash contained in the sap, as influenced by the sulphate and the chlorid of potassium. The total ash was determined by ignition and the soluble ash by extracting the ignition residue with successive portions of hot distilled water aggregating a volume of 100 cc. The alkalinity of the soluble and insoluble portions of the ash was determined by titration, using methyl orange as indicator. The calcium in the insoluble ash was determined gravimetrically. The results are presented in Table II.

TABLE II.—*Character of the ash of tobacco sap as affected by the chlorid and the sulphate of potassium*

Fertilizer treatment of tobacco.	Total ash.	Soluble ash.	Insoluble ash.	Alkalinity of soluble ash.[a]	Alkalinity of insoluble ash.[a]	CaO in insoluble ash.
Regular fertilizer test plots:	*Gr.*	*Gr.*	*Gr.*			*Gr.*
N+P	0.1694	0.0538	0.1156	0.6	22.4	.....
N+P+80 pounds K_2O, as sulphate	.1816	.1320	.0496	7.1	10.5	.....
N+P+160 pounds K_2O, as sulphate	.2219	.1748	.0471	11.4	9.4	.....
N+80 pounds K_2O, as sulphate	.1497	.1167	.0330	11.7	7.2	.....
P+80 pounds K_2O, as sulphate	.1801	.1531	.0270	10.3	5.0	.....
Special potash test plots:						
N+P	.1999	.0450	.1549	4.1	32.6	0.090
N+P+24 pounds K_2O, as chlorid	.1528	.0574	.0954	.7	20.1	.055
N+P+24 pounds K_2O, as sulphate	.1822	.0707	.1115	7.3	23.5	.061
N+P+80 pound K_2O, as chlorid	.1595	.1228	.0367	.6	8.6	.020
N+P+80 pounds K_2O, as sulphate	.1661	.1202	.0459	10.7	10.3	.028

[a] Expressed in cubic centimeters of *N/10* acid required for titration.

Both the chlorid and the sulphate of potash produce marked increases in the soluble portion of the ash of the sap and equally as large decreases in the insoluble portion. There is no marked difference in this respect in the action of the two salts, perhaps indicating that approximately equal quantities of potash are absorbed by the plant from these two sources when the rates of application to the soil are equal. That the effects of the two salts on the metabolism of the plant are different, however, is clearly shown by the alkalinity of the soluble and insoluble

portions of the ash. Roughly, the alkalinity of the soluble ash is a measure of the potash in combination with citric and malic acids in the sap, while the alkalinity of the insoluble portion represents the calcium and magnesium salts of these acids existing in solution in the sap. Both the sulphate and the chlorid greatly reduce the alkalinity of the insoluble ash, that is, the content of the sap in calcium and magnesium citrates and malates. The most striking effect, however, is the great increase in the sap content of potassium salts of citric and malic acids caused by the sulphate, as indicated by the content of soluble carbonate in the ash. The chlorid, on the other hand, is wholly ineffective in this respect. This difference in the effect of the sulphate and the chlorid on the content of soluble salts of organic acids is of practical importance, for it is known that the potassium salts of citric and malic acids improve the combustibility of tobacco (*1*).[2] On the other hand, the acid calcium salts of these acids, which largely make up the grain particles of the cured tobacco leaf (*5*), injure the combustibility, especially if the grain material is not definitely aggregated. If the organic acids are intermediate products in the respiratory combustion of carbohydrate in the plant, it appears that in some way the oxidative processes are less complete in the presence of the sulphate than when the potassium is supplied as chlorid. It is seen from Table II that addition of the potash salts to the soil lowered the calcium content of the plant sap.

It seemed possible that the unfavorable action of potassium sulphate on the vigor of the tobacco plant might be due to injurious increase in hydrogen-ion concentration of the cell sap. To test this matter leaves were collected at intervals, in the manner already described, from the special potash test plots and from other specially treated plots, using six plants on each plot for material. The leaf material was not subjected to freezing but was ground in a well-tinned meat chopper and the expressed sap was passed through muslin. The hydrogen-ion concentration of the saps thus prepared was determined electrometrically at room temperature according to the usual procedure. The results are presented in Table III.

TABLE III.—*Hydrogen-ion concentration of cell sap as affected by the sulphate and the chlorid of potassium and other salts*

Fertilizer treatment of tobacco.	Hydrogen-ion concentration of sap collected at stated intervals.				
	July 18.	July 23.	July 28.	Aug. 5.	Average.
Special potash test plots:	P_H	P_H	P_H	P_H	P_H
Control	5.47	5.40	5.26	5.26	5.35
36 pounds K_2O per acre as sulphate	5.61	5.65	5.47	5.43	5.54
36 pounds K_2O per acre as chlorid	5.43	5.33	5.29	5.28	5.33
80 pounds K_2O per acre as sulphate	5.50	5.63	5.52	5.46	5.53
80 pounds K_2O per acre as chlorid	5.64	5.50	5.46	5.29	5.48

[2] Reference is made by number (italic) to "Literature cited," p. 40.

TABLE III.—*Hydrogen-ion concentration of cell sap as affected by the sulphate and the chlorid of potassium and other salts*—Continued

Fertilizer treatment of tobacco.	Hydrogen-ion concentration of sap collected at stated intervals.			
	July 21.	July 30.	Aug. 6.	Average.
Special row tests:	P_H	P_H	P_H	P_H
Control	5.60	5.43	5.41	5.48
200 pounds NaCl per acre	5.85	5.63	5.45	5.64
Control	.....	5.60	.....	.....
200 pounds Na_2Co_3 per acre	.....	5.62	.....	.....
Control	5.79	5.86	.....	.....
1,000 pounds $CaCo_3$ per acre	5.95	5.85	.....	.....

The complete fertilizer treatments of the plots used in the observations on hydrogen-ion concentration are given on pages 29 and 31. The data given in Table III show that there is a progressive increase in hydrogen-ion concentration of the leaf sap as the leaf advances toward maturity. This progressive increase in active acidity is markedly retarded by the sulphate of potassium and to a lesser degree by heavier applications of potassium and sodium chlorids. Neither calcium carbonate nor sodium carbonate produced any marked effect. It is clear that the sulphate of potassium did not increase the active acidity and, in fact, it caused a considerable decrease in acidity. In these tests the leaves from plots treated with chlorids were free from sand drown, while the others, and especially those from the plots receiving sulphates, showed evidences of the disease. Thus, while sulphates may promote the development of a pathological chlorosis they actually retard the normal yellowing of the leaf which is associated with ripening.

The total calcium and the water-soluble sulphate (SO_4) were determined in dried leaves from the plot treated with common salt and the control plot, as well as from the plots receiving 80 pounds potash per acre as sulphate and chlorid, respectively. The results are shown in Table IV.

TABLE IV.—*Influence of sulphates and chlorids on total calcium and water-soluble sulphate in the dried leaf*

Fertilizer treatment of tobacco.	Total ash in leaves.	Total calcium in leaves.	Water-soluble sulphate (SO_4) in leaves.
	Per cent.	*Per cent.*	*Per cent.*
Control	13.06	3.46	1.66
200 pounds NaCl per acre	12.09	1.74	.67
80 pounds K_2O per acre as chlorid	8.89	1.40	.25
80 pounds K_2O per acre as sulphate	10.50	1.36	1.50

It seems likely that the greatly increased growth of the plant resulting from the use of the sodium chlorid is largely responsible for the decrease in percentage content of calcium and soluble sulphate. The calcium content is the same in the plots treated with the sulphate and the chlorid

of potassium, and since sand drown was present on the plants receiving the sulphate the disease can not be due to calcium deficiency. The increased content of soluble sulphate, however, is undoubtedly significant.

No evidence of soluble oxalates could be found in the sap from leaves affected with sand drown.

MAGNESIUM DEFICIENCY THE CAUSE OF SAND DROWN

As previously stated, the effectiveness of magnesium sulphate in preventing sand drown in field tests at Oxford while other sulphates greatly aggravated the trouble suggested that lack of an adequate supply of magnesium might be the cause of the trouble. Pot tests soon proved this to be the case. The cultures were conducted in 12-quart galvanized-iron buckets with perforated bottoms, using sassafras fine sandy loam soil from Prince Georges County, Md., and in some cases the Durham sandy loam soil from the Oxford (N. C.) Tobacco Station. As a complete nutrient solution for the cultures a mixture of 10 gm. sodium nitrate, 5 gm. monocalcium phosphate, 5 gm. potassium sulphate and 5 gm. magnesium sulphate was dissolved in 10 liters distilled water. In some cases potassium chlorid was substituted for the sulphate and ammonium nitrate was used in place of the sodium salt. For one series in each test the magnesium sulphate was omitted from the nutrient solution. To accentuate the effect of omitting magnesium the plan was adopted of watering the plants with the nutrient solution instead of distilled water, the solutions being added in considerable excess so as to cause a leaching effect. This plan proved to be quite successful. Where magnesium was omitted from the solution sand drown quickly appeared. A typical case in the advanced stage is shown in Plate 4. It will be seen that the leaves have lost nearly all color and hang limp, the cells having lost their turgidity. It is to be noted, however, that seed formation was successfully accomplished and the chlorotic leaf tissue remains alive for many days. Under favorable conditions the disease may be induced by applying the fertilizer salts (with magnesium omitted) to the soil before transplanting and avoiding any leaching action in watering the plants, as is shown in Plate 5. Naturally, the symptoms are less severe than when leaching is employed. When the disease has not progressed too far it is readily cured by addition of magnesium to the soil. A case of this sort is shown in Plate 6. The plant at the right, affected with sand drown in severe form, to which magnesium sulphate had been subsequently added was rapidly recovering at time of photographing. Thus, while soluble sulphates generally will greatly aggravate sand drown, sulphate of magnesium both prevents and cures the malady. It seems proper, therefore, to consider lack of an adequate supply of magnesia as the primary cause, whatever may be the internal processes involved. On the other hand, the importance of an increased supply of soluble sulphate as an inciting factor should not be overlooked. In other words, the ratio of magnesia to soluble sulphate seems to be of prime importance.

SAND DROWN CONTRASTED WITH OTHER TYPES OF CHLOROSIS

The tobacco plant with its extensive foliar expanse is well adapted to the study of pathological symptoms such as those characteristic of sand drown; and the plant, in fact, is subject to several types of chlorosis. Of special importance in this connection is the chlorosis due to potash hunger. In typical cases, the symptoms of potash hunger are readily

distinguished from those of sand drown, though the two diseases may be confused in casual examination. In both cases the chlorosis begins at the tips of the leaves and on the margins, and the lower leaves are affected first. In extreme cases of sand drown there may be some distortion of the leaf margin, but ordinarily the surface is smooth and the leaf attains full size. In potash hunger the leaf is puckered and presents an uneven surface because of the difference in rate of growth of the veins and the leaf web. Arrested growth of the periphery causes a characteristic curving downward of the margin, and, particularly, the tip of the leaf. The farmer speaks of the leaf as being "rim bound." Chlorosis due to potash hunger is promptly followed by the appearance of small dead spots in the affected portions of the leaf. The initial stages of this spotting are shown in Plate 7. As the malady progresses large areas of the leaf die, especially along the margins which frequently become ragged and torn. This condition is popularly spoken of as "rimfire." This localized dying of the leaf tissue in potash hunger is an important aid in distinguishing the trouble from sand drown. Finally, the destruction of the green color of the leaf in potash hunger gives rise to a dull yellow, with a bronze or copper overcast, whereas sand drown results in a very light yellow or cream color. In severe potash hunger the green portion of the leaf shows a dark "muddy," bluish green shade, while in sand drown the non-chlorotic portion of the leaf shows the normal green color.

In sulphur deficiency the entire leaf, including veins and midrib, as well as the stem of the plant, is uniformly affected, the color of the whole being a light shade of green. This color is frequently shown when muriate of potash is used instead of the sulphate as fertilizer. When both sulphur and magnesia are deficient the entire plant becomes more distinctly chlorotic, the color being suggestive of that characteristic of the White Burley variety of tobacco as it approaches maturity. As is well known, a limited nitrogen supply also results in a plant with leaves of a light shade of green. In the disease known as frenching there is a partial chlorosis of the leaves, but there are also morphological changes, the size and especially the width of the leaf being reduced through failure of the leaf lamina to expand normally. In the infectious chlorosis called mosaic there is a characteristic mottling distributed rather uniformly over the leaf and appearing only in growing tissues; hence it is easily distinguished from sand drown.

MAGNESIA AS A FACTOR IN PRACTICAL FERTILIZER USAGE

It has been recognized for many years that magnesium is one of the indispensable elements of plant food, and Willstätter (7) has demonstrated that it is an essential constituent of chlorophyll. A considerable number of experiments have been made concerning the fertilizing value of magnesium compounds, the results of which are more or less contradictory. Few of these tests, however, have been so planned as to throw light on the present problem, and in most cases the action of magnesium salts has been regarded as being indirect, resulting in the liberation in the soil of other plant food elements (2). In pot experiments with one of two soil types studied, Wheeler and Hartwell (6) obtained increased growth of barley and rye by the use of magnesium sulphate and chlorid, and it is suggested that the favorable effect was due, at least in part, to a direct nutrient action of the magnesium. Recently A. Jacob (3) has called attention to favorable results obtained by the German Kali Syndicate in the use of potash salts containing magnesia as fertilizer for pota-

toes. Considerable attention also has been given to the toxic effects of magnesia compounds when present in excess, especially since Loew developed his well-known theory of optimum lime-magnesia ratios for crop plants (*4*).

It seems clear that on many soils it is essential for normal development of the tobacco plant that the fertilizer carry a certain minimum quantity of magnesia, especially when the fertilizer contains soluble sulphates. Preliminary observations indicate that corn and cotton and probably other crops have similar requirements. The exact needs of the tobacco crop will require considerable study, since both the yield and the quality of the product are involved. The combustibility of tobacco is affected adversely by salts of magnesia, and it appears that the moisture-holding properties, and therefore the elasticity, of the leaf are influenced by the magnesia supply. The highly injurious effect of magnesium deficiency on the color of the leaf has already been discussed. Hence, it seems likely that any considerable excess of magnesia, as well as a deficiency, must be avoided for best results.

Detailed data of plot yields with tobacco in different localities for a period of years comparing the action of sulphate and chlorid of potash, which involve the effects of magnesium deficiency, are reserved for publication elsewhere. It will suffice to present here the results obtained at Oxford, N. C., in 1920, as illustrating the striking effect of magnesium deficiency on the market value of the crop. The detailed fertilizer treatment of these plots has already been described. One-half of each plot received ground limestone containing magnesia applied broadcast in the fall of 1919 at the rate of 1 ton per acre. The yield and value of the tobacco on each plot are shown in Table V.

TABLE V.—*Yield and value per acre of tobacco receiving sulphate and muriate of potash, with and without addition of ground limestone, Oxford, N. C., 1920*

Potash (K_2O) added as fertilizer.	Yield of tobacco.		Value of tobacco.	
	Without lime.	With lime.	Without lime.	With lime.
	Pounds.	*Pounds.*		
None	330	450	$27	$38
12 pounds per acre, as sulphate	260	660	33	105
12 pounds per acre, as muriate	520	640	67	126
24 pounds per acre, as sulphate	480	620	47	93
24 pounds per acre, as muriate	620	820	107	147
36 pounds per acre, as sulphate	600	640	88	90
36 pounds per acre, as muriate	780	780	156	117
80 pounds per acre, as sulphate	700	580	78	107
80 pounds per acre, as muriate	800	920	115	218

Considering only the treatments without lime, it is seen that the muriate gives far better results than the sulphate; and the difference becomes most striking with the intermediate rates of application. The injury in quality of the tobacco from the use of sulphate is proportionately greater than the reduction in yield as compared with the muriate. Evidence has already been given that addition of magnesia fully overcomes the unfa-

vorable action of the sulphate. Results fully as remarkable as the foregoing have been obtained by Mr. E. H. Mathewson, of this office, in similar cooperative tests at Reidsville, N. C.; and the experiments elsewhere indicate that the data will apply to the tobacco crop more or less generally.

Muriate of potash tends to impair the combustibility of tobacco and in some cases gives less desirable colors. It seems desirable, therefore, to use the sulphate of potash in combination with sulphate of magnesia. Tests have shown that low-grade potash salts carrying more or less magnesia, such as Kainit and double manure salts, eliminate sand drown and produce much better growth than high-grade sulphate. It has been found, in fact, that both muriate and sulphate of potash in relatively pure form but still containing very small percentages of magnesium salts, such as some of the "high-grade" imported potash salts, are less likely to cause sand drown than muriate and sulphate of exceptional purity. It seems clear that very pure forms of potash salts should not be used as fertilizer for tobacco, and possibly not for other crops, unless supplemented with magnesia salts or materials containing magnesia. In the absence of more exact data double manure salts, which are essentially mixtures of sulphate of potash and sulphate of magnesia, would seem to be a desirable source of potash for tobacco. Wood ashes, cottonseed-hull ashes, and other forms of potash of vegetable origin also should be satisfactory so far as concerns magnesia.

It is to be noted that in actual fertilizer practice high-grade sulphate of potash has not been used very extensively in this country, and even when employed it is nearly always in combination with other fertilizer materials which contain magnesia. It is believed that the favorable action of cottonseed meal, tobacco stems and stalks, and barn manure in preventing sand drown, which has been previously discussed, is due to the magnesia supplied by these materials. Cottonseed meal contains about 1 per cent magnesia, and this offers a possible explanation of the marked preference for this fertilizer material shown by tobacco growers of the Connecticut Valley where color of the cured leaf is of so much importance. It may be that vegetable material of this character through gradual decay in the soil furnishes a more or less continuous source of magnesia in suitable quantities through the growing season. Thus, it seems likely that one of the functions of barn manure is to furnish magnesia in suitable quantity and form for plant growth. The action of common salt in preventing sand drown, which has been referred to, probably is due in part to the small percentage of magnesia which it usually contains, but it is also likely that salt liberates magnesia when applied to the soil.

LIME IN RELATION TO SAND DROWN

While limestones vary widely in their content of magnesium, it rarely happens in practice that limestone or burnt lime available for agricultural uses is entirely free from this element. The available evidence all goes to show that the quantity of magnesium required to prevent the sand-drown type of chlorosis is small, possibly less than 20 pounds per acre. In general, therefore, it is to be expected that liming the soil even with fairly pure limestone or its products would prevent sand drown, keeping in mind, of course, that the magnesia thus supplied would be less soluble than that furnished by most of the impure potash salts.

The data given in Table V plainly show that liming is decidedly beneficial in overcoming the tendency of sulphate of potash to cause

sand drown, and heavier applications probably would further increase the effectiveness of both the sulphate and the chlorid of potassium by removing magnesium deficiency as a limiting factor. It is of interest to note that Wheeler and Hartwell (*6*) found a marked increase in content of magnesium but not of calcium in both red clover and common sorrel (Rumex) grown on soil which had been limed. The value of liming in overcoming sand drown has been confirmed in other tests involving the use of various potash salts containing different percentages of magnesia. In all cases sand drown associated with the use of the purer potash salts was entirely prevented by applying dolomitic limestone. Thus, to the various functions in improving soil productiveness which have been ascribed to liming must be added that of preventing or correcting magnesium deficiency. Again, it has been shown that one of the harmful effects which may result from the use of ammonium sulphate as fertilizer without liming is to cause a deficiency of magnesium, and this injurious effect may be remedied by use of magnesium salts as well as by liming. It is to be noted that the value of limestone in supplying small quantities of magnesia has little or no connection with the theory of optimum lime-magnesia ratios.

NEW METHOD FOR CONDUCTING POT CULTURES IN THE STUDY OF FERTILIZER PROBLEMS

It is well known that it is difficult in most cases to harmonize the results of pot tests conducted under accurately controlled conditions with results obtained in the field. Experience obtained in the study of sand drown has emphasized two important particulars in which the two types of experimentation usually differ. In the first place, chemically pure salts are generally used in pots cultures while in the field pure fertilizer salts are practically never used. In the second place, in conducting pot tests precautions are usually taken against any loss of material through drainage, while in the field more or less leaching invariably occurs. By common consent investigators and farmers alike constantly speak only of the three elements, nitrogen, phosphorus, and potassium (with occasional reference to sulphur) in discussing fertilizers. In practice, however, the use of so-called complete fertilizers regularly involves applying to the soil at least six essential elements, namely, nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium. It is not surprising, therefore, that in ordinary fertilizer usage the need of the three last-named elements does not become apparent. It has been shown, however, that the result is very different, at least in the case of tobacco, when fertilizer salts of high purity are used. On sandy soils subject to severe leaching and containing little organic matter it should not be surprising to find a deficiency of magnesium or sulphur or calcium as well as of nitrogen, phosphorus, or potassium. Sand drown seems to furnish a convincing illustration.

The satisfactory results obtained in inducing sand drown in pot cultures have suggested that by giving due consideration to commercial fertilizer salts with respect to all of the six elements mentioned and by providing for leaching effects pot cultures can be made to yield results more in line with field experience. The essential feature of the leaching process consists in the use of nutrient solutions, complete and with one or more elements omitted, as desired, instead of distilled water for producing leaching effects. The leaching can be pushed as far as desired without

impoverishing the soil except with respect to the elements omitted in the nutrient solution. Obviously the method can not well be applied to compact, clayey soils.

CONCLUSION

Sand drown, a chlorosis of tobacco involving both the green and the yellow pigments of chlorophyll, is due to magnesium deficiency and is markedly aggravated by an increased sulphur supply. For these reasons the use of potash salts of high purity (free from magnesia) and, especially, pure forms of the sulphate have resulted in serious damage to the crop. The need for magnesium is readily met by the use of sulphate of potash containing magnesia or by applying lime containing magnesia. One of the functions of organic matter of vegetable origin, as cottonseed meal, tobacco stems, and barn manure and of lime, is to furnish small quantities of magnesia to the plant through the growing season. The sand-drown disease of tobacco suggests that in interpreting fertilizer action the sulphur, magnesium, and calcium commonly contained in "complete" commercial fertilizers should be taken into account as well as the nitrogen phosphorus, and potassium. A method of conducting pot cultures in which the soil is leached with complete and incomplete nutrient solutions as desired, gave good results in the investigation of sand drown and is suggested for use in the study of problems in fertilizer action. It remains to be determined to what extent the findings concerning sand drown of tobacco will apply to other crop plants.

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PLATE 1

Tobacco plant of flue-cured type affected with sand drown. Note that the plant as a whole and the individual leaves have attained normal size and shape and the leaf surface is relatively smooth. The disease begins in the lower leaves and at the tips of the leaves. The veins retain their green color long after the leaf lamina becomes bleached. The leaf lamina usually does not die in local areas or spots as in potash hunger.

PLATE 2

Leaves of tobacco of flue-cured type affected with sand drown, showing successiv
stages of the disease. The leaf at the bottom has lost practically all green color excep
along the larger veins and at the base. From bottom to top these leaves fairly repr
sent contemporaneous stages of advancement of the disease on different leaves of th
same plant from the base upward.

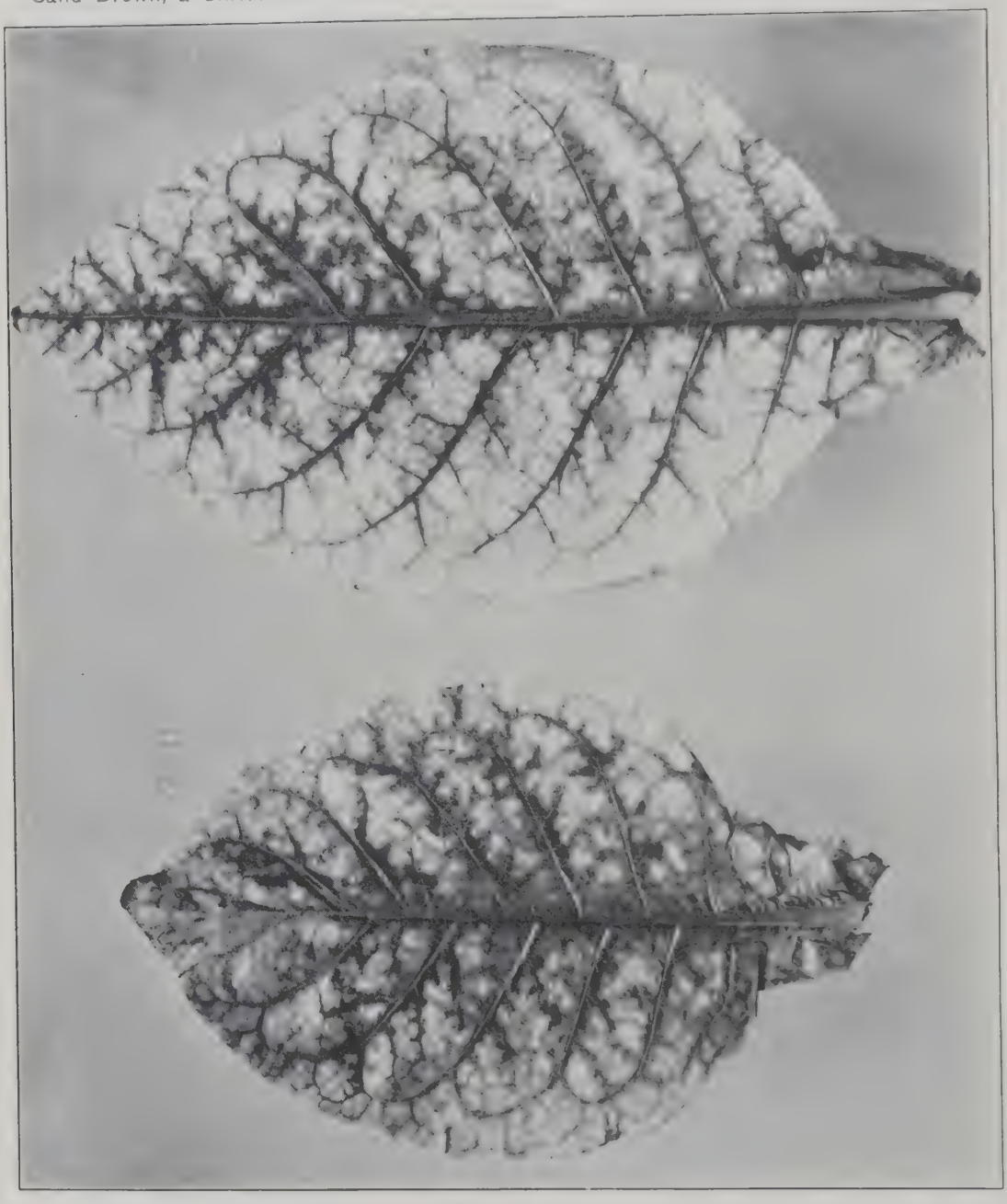

PLATE 3

Leaves of tobacco of cigar-wrapper type severely affected with sand drown, the leaf at the top showing an advanced stage of the disease. Specimen collected at Hatfield, Mass., by Dr. James Johnson.

PLATE 4

Plant at right, which is normal, was watered with the complete nutrient solutio described on page 35, including magnesium sulphate, the solution being added i excess so as to produce a leaching effect. Plant at left received the same treatment i all respects, except that magnesium sulphate was omitted from the nutrient solutio Note the advanced stage of sand drown, in which the leaves hang limp. Note, hov ever, that the leaf tissues remain alive and seed formation is successfully accomplishe

PLATE 5

The two plants received the same treatments as the two plants shown in Plate 4, except that the plant nutrients were applied to the soil before setting the plants and the plants were watered with distilled water in limited quantities so as to avoid any leaching action. The plant at left, which received no magnesia, shows pronounced symptoms of sand drown but in less severe form than that of the corresponding plant in Plate 4.

PLATE 6

The two tobacco plants shown were watered with an excess of a nutrient solution containing no magnesia, so as to produce a leaching effect. After well-defined symptoms of sand drown had developed, magnesium sulphate was added to the nutrient solution applied to the plant at right, while the plant at left continued to receive the magnesium-free nutrient solution. At time of photographing plant at right was rapidly recovering. Note the turgidity of the leaves and the partial restoration of the natural color.

PLATE 7

Tip of tobacco leaf, showing chlorosis due to potash deficiency. Note that there are numerous small spots which represent dead tissue. This spotting of the leaves due to localized dying of the tissues is a characteristic feature of potash deficiency symptoms as distinguished from the symptoms of sand drown.

PARASITISM OF SCLEROTIUM ROLFSII ON IRISH POTATOES [1]

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INTRODUCTION

According to various published statements *Sclerotium rolfsii* Sacc. attacks a great number of hosts.[2] It has been frequently reported on the Irish potato, though a clear and full description of the symptoms of its effect upon this host has not yet been given. Sometimes the disease has been designated as blight, in other cases as wilt, and very little has been said about the tuber-rot. The type of the decay, as distinguished from other tuber decays, has not been satisfactorily defined, and the mode of the destruction of the host cells has not been followed in detail. In view of this scarcity of information the writers feel justified in presenting certain of their observations and experiments relative to this subject.

NATURAL INFECTION

Both the vine and the tuber of the Irish potato may be attacked by this fungus. The disease of stem and foliage may manifest itself in several different forms which vary with the age of the plant and with the environmental conditions. If very young plants are attacked and the soil moisture is abundant, they are most likely to show damping-off symptoms. Older plants may suffer from a rootrot or a stemrot or from both with subsequent wilting and drooping of the leaves and the stems, which ultimately lie prostrate on the ground. (Pl. 1, A.)

Natural infection in the field was observed in the South in advanced stages in several States. The symptoms produced were in general those of wilt or stemblight. The disease had evidently made its attack at or near the surface of the soil, causing a decay of the stem at that point and for a little distance above and below. Frequently the stem was consumed below to such an extent that little of the underground tissues except a few strands of vascular fiber remained attached if the tops were lifted. Wefts of mycelium or the sclerotia of the fungus could be seen clinging to the stem or extending radially from the plant in and on the surface of the soil.

ARTIFICIAL INOCULATION

To obtain further evidence in regard to the symptoms of the disease certain field experiments were conducted with artificially inoculated tubers. These experiments were carried on in 1919 and 1920 at Arlington

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[2] TAUBENHAUS, J. J. RECENT STUDIES ON SCLEROTIUM ROLFSII SACC. *In* Jour. Agr. Research, v. 18, no. 3, p. 127–138, 1 fig., pl. 3–6. 1919. Literature cited, p. 137–138.

Farm, Va., with two varieties of potatoes, Irish Cobblers and Bliss Triumphs, and two strains of the fungus. One of these latter, culture No. 126,[3] was isolated in July, 1918, from a decaying potato tuber grown in North Carolina and the other, culture No. 127, in May, 1918, from a diseased potato stem grown in Arkansas. The diseased material from both sources represented clear cases of the Sclerotium infection.

Ten tubers of each variety were inoculated in 1919 with Sclerotium No. 126 and 10 with Sclerotium No. 127, making a total of 40 inoculated tubers. In addition, a number of uninoculated tubers of both varieties were planted for control. All these potatoes were grown the preceding season in Aroostook County, Me., where sclerotium-rot has never been found. The inoculations were made a few days before planting, a sclerotium or two being inserted in a narrow channel made in each tuber by means either of a cork borer or a needle. When the potatoes were taken to the field a few millimeters of decay could be noted in every tuber on cutting. The planting was done on May 19, following a period of rainy weather. There was an abundance of moisture in the soil although the surface of the ground was reasonably flat. The two months following the planting were characterized by prolonged warm and dry weather, intermittent with two rainy periods.

The effect of *Sclerotium rolfsii* on growing potatoes in this test was extremely disastrous. Part of the plantings did not show above the ground at all, part perished from damping-off, and most of the remainder gradually wilted and died before maturity. The progress of the disease during the season was as follows:

June 10. Number of missing hills	11
June 10. Number of small plants (1 to 3 inches) damped-off	7
June 18. Number of larger plants showing wilt	10
June 24. Additional number of wilting plants	6
July 14. Additional number of wilted plants	1
Total number of missing and wilted plants	35

The remaining five plants, although considerably weakened, remained alive until harvest. Their size was only about half that of the plants in the control row, but the foilage and the stems were practically normal in appearance (Pl. 1, B). It is probable that the action of the parasite in these cases was confined to the root system alone.

At digging time, on September 15, no tubers were found in any of the 35 hills wilted or otherwise destroyed by the fungus. The other 5 hills produced a few tubers of the size of a hazel nut, but no rot was in evidence. Control rows produced medium-sized sound tubers, and they showed no signs of damping-off or wilt throughout the growing season.

There was no perceptible difference in the response of the two varieties of potatoes used. However, the severity of the attack of the two fungus strains differed somewhat in this experiment. Thus on June 24 (five weeks after planting), all plants inoculated with Sclerotium No. 126 were either dead or showed distinct symptoms of wilt, while among those inoculated with Sclerotium No. 127, six plants still were apparently unaffected, three of each variety. On July 14 wilt appeared in an additional Bliss Triumph plant; but the other five plants, three Irish Cobblers and two Bliss Triumphs, while under size, showed no other signs of the disease and remained alive as long as the control plants.

[3] Numbers of the cultures in this paper have reference to the writers' collection.

The various symptoms of the disease followed each other in succession. Some seed tubers decayed so rapidly that the sprouts never reached the surface of the ground. Thus, the missing hills, a little over 25 per cent of the total number planted, gave the first indication of the activities of the parasite. About 16 per cent of those that did come up were able to form only a few first leaves, as the stems became thoroughly infested with the fungus, turned dark and water-soaked at and a little below the surface of the ground, and died. The appearance of such plants was fully typical of damping-off. The next distinct stage of attack may quite properly be characterized as wilt. It was observed with older plants, when not only the roots but also the stems were so seriously injured by the parasite that the activity of the conducting vessels was greatly impaired. A little later the stems were so corroded that the plants died. In some cases the foliage symptoms were so typical of wilt that it would be difficult on casual observation to distinguish this form from those caused by Fusarium, Verticillium, or bacteria (Pl. 2, A). It is very easy, however, to ascertain the actual cause of the trouble on closer examination of the base of the stem, where usually a weft of white mycelium and the brown sclerotia of *Sclerotium rolfsii* can be found as well as a stemrot in place of the discoloration of the vascular system. In some cases the infestation of the stem was not deep enough to sever completely the vascular connections; and then the symptoms of the wilt were not so pronounced, but the plants presented a general unhealthy appearance with partly drying, partly wilting foliage. This form may readily come under the conception of blight. This condition, too, led to the ultimate death of the plants (Pl. 2, B). The number of wilted and blighted plants in the foregoing experiment constituted a little over 40 per cent.

A repetition of this experiment was made in 1920 on another piece of land on the same farm. Only the Irish Cobbler variety was used this time, but there were two sets of plantings—an early one, on April 8, and a later one, on May 6. Twelve hills to each strain of the fungus in each series were planted, or 48 hills in all. The remainder of the procedure was identical with that of 1919. The progress of the disease was one of great interest. Up to the middle of June all plants in the earlier set inoculated with culture No. 126 remained healthy, and of those inoculated with culture No. 127 two showed wilt. On the same date in the later set there were 2 hills missing and 7 wilting in the lot inoculated with culture No. 126, while not a single hill inoculated with culture No. 127 showed any sign of infection. During the next two weeks the disease made a general advance in all the sets, and the conditions on June 29 were as follows:

EARLY PLANTING

(a) Inoculations with culture No. 126: 4 hills showed infection.
(b) Inoculations with culture No. 127: 7 hills more or less infected, in some hills only one stem showing the disease.

LATER PLANTING

(c) Inoculations with culture No. 126: 10 hills were affected, of which 5 decayed completely and disappeared.
(d) Inoculations with culture No. 127: No hill showed infection.

At harvesting time, on July 20, 3 hills remained alive in lot (a), an increase of 75 per cent in affected plants, 6 in lot (b) showing that 1 hill, partially affected, had recovered during the last three weeks before the harvest, 4 in lot (c), indicating another partial recovery, and 11 in lot (d),

which signifies death of one plant only. The general succession of the various manifestations of the disease was essentially the same as in the first test and varied with the age of the plants. Thus, there were first some missing hills in those cases where the seed pieces were destroyed promptly in the ground, then damping-off symptoms showed on the young plants, and finally wilt, stemrot, and blight followed in older plants. As to the tuber production and tuber-rot, the following conditions were observed on the date of digging:

EARLY PLANTING

Lot (a): Only 9 hills produced tubers, of small size; a severe rot in 3 hills.
Lot (b): Every hill produced tubers, all undersized; severe rot in 4 hills.

LATER PLANTING

Lot (c): Only 5 hills produced tubers, undersized; severe rot in 1 hill.
Lot (d): Tubers in every hill, varying in size; severe rot in 4 hills.

It appears from the foregoing data that the most serious infection with Sclerotium No. 126 took place in the later planting or later in the season in the earlier planting and, quite the contrary, with Sclerotium No. 127 the most serious infection took place in the earlier planting and very little infection was observed in the later planting. These results indicate, therefore, that the Arkansas strain of the fungus is more adapted to cooler temperatures, while the North Carolina strain is more adapted to warmer temperatures.

The total number of hills which became infected with culture No. 126 in both plantings in this experiment was 19, and the total number of hills which became partially or wholly infected with culture No. 127 in both plantings was only 8. This apparently weaker pathogenicity of the latter strain is quite in accord with the results obtained during the preceding season.

EXISTENCE OF STRAINS OF SCLEROTIUM ROLFSII

The markedly different parasitic activities of the two Sclerotium cultures and their different behavior in the field at different time of the season obviously suggest the existence of strains in *Sclerotium rolfsii*. Taubenhaus [4] summarizing the results of his recent work, stated that—

There are no varietal nor physiological strains in *Sclerotium rolfsii*.

It is self-evident, however, that this assertion may be regarded as correct with reference only to those strains which were included in his studies and that the generalization of the characteristics of these strains is not at all warranted. As it is seen from the foregoing account of the writers' experiments, their two strains reveal a decided physiological difference. Furthermore, these two strains exhibit a distinct morphological difference, namely, in the size of the sclerotia, as shown in Plate 3, A to C. The individual sclerotia vary in size within the strain, and their average size may be larger or smaller, depending on the kind of medium and environmental conditions; but when considered relatively, on the same medium, at the same age, grown under the same environment, the sclerotia of the culture No. 126 are always larger than the sclerotia of the culture No. 127. Besides, there is a peculiar tendency in the strain No. 126 when food supply is abundant to mass up the sclerotia in large clusters (Pl. 3, C) which has not been noted in the "microsclerotial" strain No. 127. The sclerotia in the clusters are often of a much larger diameter than the individual sclerotia and tend to become elongated.

[4] TAUBENHAUS, J. J. Op. cit.

curved, or irregular in shape. The constant relative difference in the size of the sclerotia of the two strains was observed on the following media: Raw potato, cooked potato plugs, potato agar, corn-meal agar, oatmeal agar, rice agar, and rice. Other media were not included in the studies.

Considering the characters of the two cultures stated in the preceding paragraphs, the writers believe the conclusion to be quite warranted that the difference between Sclerotium No. 126 and Sclerotium No. 127 is that of, at least, varietal character. Having no authentic culture of *Sclerotium rolfsii* Sacc. they are unable to determine which, if either, of the two is the true species.

CHARACTER OF SCLEROTIUM TUBER-ROT

The potato tubers infected with *Sclerotum rolfsii* in the field or artificially inoculated in the laboratory are subject to a rapid progressive decay. This decay is practically odorless and colorless in its earlier stages but takes on a yellowish appearance in older portions, particularly if there is a considerable development of the fungus hyphae. The affected host tissues become usually more or less porous. This was exactly the type of rot from which culture No. 126 was isolated. It was also repeatedly reproduced in laboratory inoculations. The term "white rot" would seem to be quite appropriate to and descriptive of this stage of the disintegration. Under sufficiently favorable moisture and temperature conditions the decay may develop into what has been termed "melter" type, when the affected portions become exceedingly soft and watery.

Most of the laboratory inoculation experiments were carried on with the Irish Cobbler variety, but a small quantity of Bliss Triumph and a seedling variety were also tested with no apparent difference in the response of these varieties to the action of the parasite. Inoculum was inserted either in needle pricks or in shallow scalpel wounds. Both relatively young sclerotia and young mycelium of the fungus were used with practically the same success. The inoculated tubers were placed in ordinary glass moist chambers or stone jars with layers of wet filter paper below and above and were left in the laboratory for two weeks at the temperature of 20° to 22° C. The difference in extent of rot produced under these conditions by the two Sclerotium cultures was only slight and may not alone be regarded as significant. The diseased areas resulting from inoculation with culture No. 126 averaged 23 to 25 mm. in diameter and 14 to 15 mm. in depth; those resulting from inoculation with culture No. 127 averaged 18 to 20 mm. in diameter and 10 to 12 mm. in depth. The "melter" type of decay did not develop during this period and under these conditions. Liquid, however, separated from tubers kept in moist chambers for a longer period of time.

The destructive effect of the fungus on potato tuber tissue was very clearly shown when sterile raw potato blocks were inoculated in large quantities in Erlenmeyer flasks. There first developed an abundant growth of pseudo-parenchymatous mycelium emveloping the blocks and practically filling the flasks. Abundant sclerotial formation followed with the development of large compact aggregates of these bodies which were frequently an inch or even more in diameter. When the mycelial development had reached its maximum there appeared a gradually increasing accumulation of light amber liquid in the bottom of the flask, sometimes amounting to as much as 500 cc. in a single 2-liter flask. Since no additional water was placed in the flask with the raw potato blocks, it is evident that this liquid resulted from the action of the fungus

on the potato. Observations made at various stages in the process showed that the mycelium enveloped but did not penetrate the blocks. The middle lamellae first softened, then the cell contents and cellulose walls. The starch grains were corroded and eventually entirely dissolved. If the mycelial mass surrounding the space originally occupied by an individual block was broken open at the proper stage it was found to inclose a cavity approximately the size and shape of the original block but containing nothing but a residue of liquid. Earlier stages showed free starch granules or disintegrating tissue. The starch appears to be the last of the solids to disappear. If the cultures were allowed to remain undisturbed for a sufficient period, at least partial autolysis of the mycelium and sclerotia occurred and the flasks instead of being practically filled contained a more or less compact aggregate of mycelial slime and embedded sclerotia of 200 or 300 cc. volume nearly submerged in a few hundred cubic centimeters of brown liquid.

Examination of the raw potato blocks at various stages of disintegration indicated that the fungus accomplishes its work without penetrating the tissue. The logical inference is that digestive enzyms are secreted which dissolve the host tissue and the digested material becomes available to the fungus by diffusion and osmosis. Taubenhaus [5] made no special study of the enzymic activity of the fungus but states that—

> infection seems to be favored by an emzym secreted by the advancing mycelial strands. . . . Fungous hyphae are not found within the cells but only between them where the middle lamella has disappeared.

Under the artificial conditions of the writers' flask experiments even such mycelial penetration appeared confined to a very shallow surface layer or failed entirely.

Enzym preparations of the hyphae obtained by the acetone-ether method and enzym-bearing powders obtained from the amber liquid by the alcohol precipitation method were used in studying the action of the fungus. Disks of raw potato in water were treated under aseptic conditions with each of the enzym preparations as well as with filtered water extracts of each of them. Such disks invariably softened to the consistency of curd, whereas control disks similarly handled but with the addition of no enzym-carrying preparation or extract remained firm. Similar results were obtained when toluene was used as an antiseptic without special precautions against the entrance of organisms. The disks disintegrated through the softening of the middle lamellae and the consequent freeing of the individual cells, just as may be seen in the initial stages of decomposition in the presence of the fungus itself.

SUMMARY

(1) Infection of the Irish potato plant with *Sclerotium rolfsii* Sacc. may manifest itself in a variety of symptoms which, according to their appearance, may be grouped as seed-piece rot, damping-off, stemrot, wilt, and blight.

(2) Infection of the potato tuber results in a progressive soft white rot, which may take on a so-called "melter" form with profuse extrusion of liquid.

(3) The destruction of the host tissues may be accomplished without hyphal penetration by means of digestive enzyms.

(4) Existence of varietal strains in the fungus known as *Sclerotium rolfsii* is quire apparent from the physiological and morphological differences of the cultures studied by the writers.

[5] TAUBENHAUS, J. J., OP. CIT., p. 132-133.

PLATE 1

Sclerotium rolfsii on potato:

A.—Wilt and stem decay at the base from artificial inoculation of seed tubers.
B.—A dwarfed plant grown from an inoculated seed tuber.

B
A

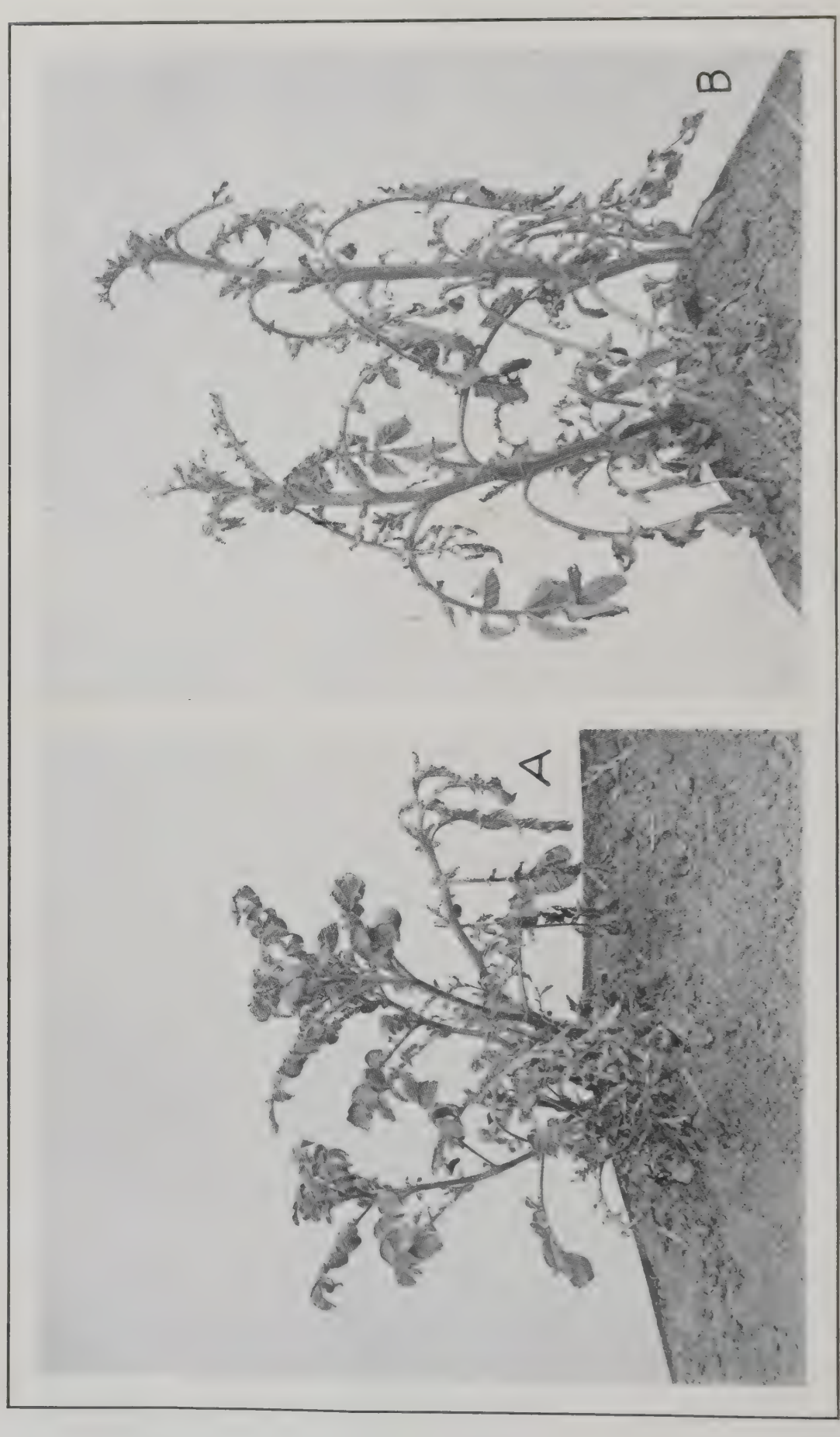

B
A

PLATE 2

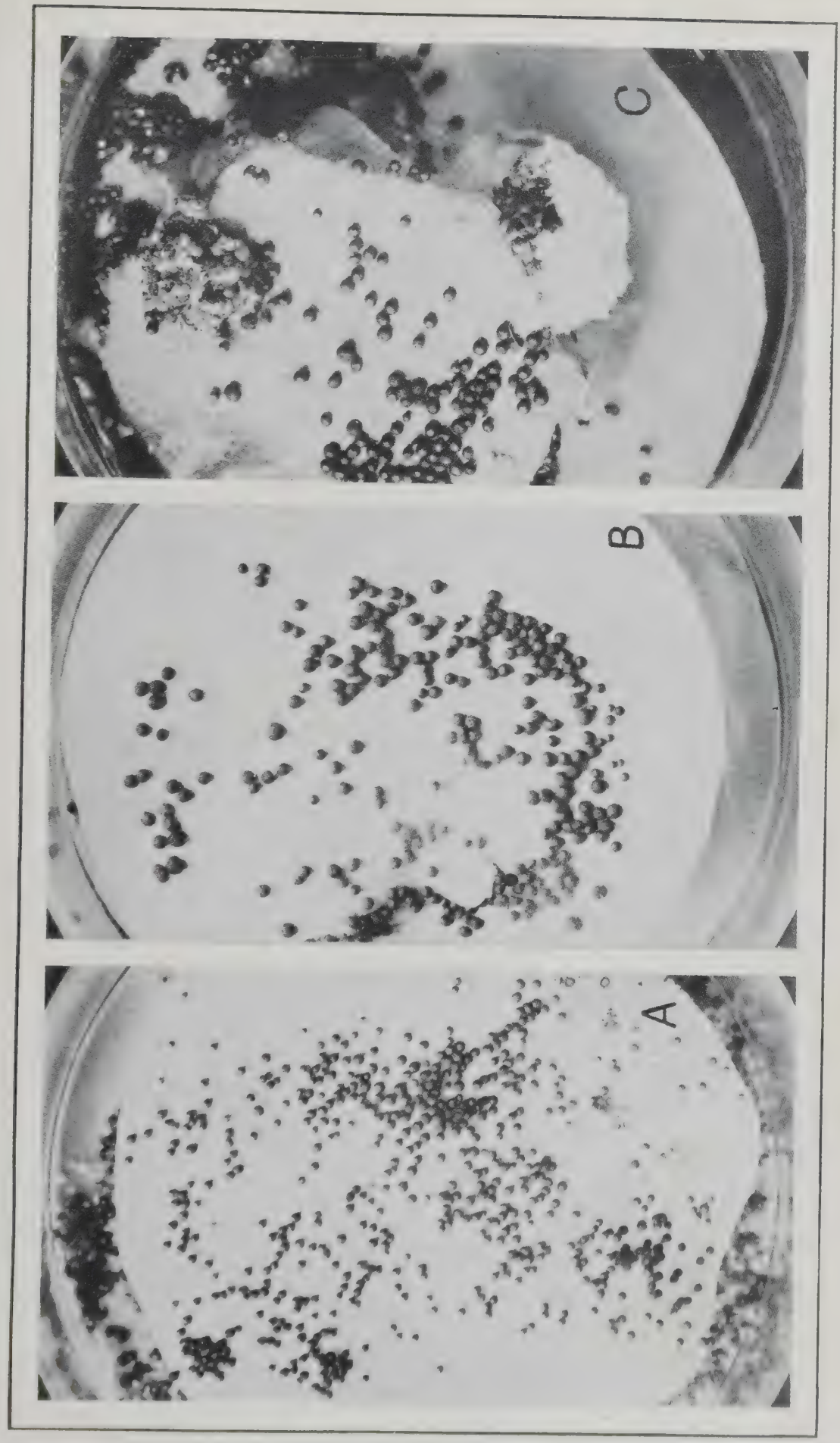
Sclerotium rolfsii on potato:

A.—Typical symptoms of wilt, result of artificial inoculation.
B.—A severe blighting condition, result of artificial inoculation.

PLATE 3

Cultures of *Sclerotium rolfsii* on raw potato slices covered with sterile filter paper.

A.—Culture No. 127 from a diseased potato stem grown in Arkansas.
B, C.—Culture No. 126 from a diseased tuber grown in North Carolina.
C.—Clusters of the sclerotia.


C
B
A

EXAMINATION OF AUTHENTIC GRAPE JUICES FOR METHYL ANTHRANILATE[1]

By FREDERICK B. POWER, *Chemist in Charge*, and VICTOR K. CHESNUT, *Assistant Chemist, Phytochemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

In a preliminary communication by the present authors [2] attention was directed to the occurrence of methyl anthranilate in grape juice, and it was then observed that this compound is contained to the largest extent in juices of the Concord type, although the quantity present in a number of other varieties was sufficient to permit of detection by the delicate test employed.[3] Through the kind cooperation of Dr. J. S. Caldwell, of the Bureau of Plant Industry, United States Department of Agriculture, we have been provided with a large number of samples of genuine grape juices which were prepared from definite varieties of the fruit and in different sections of the country. It has thus been made possible for us to obtain further information respecting the distribution of methyl anthranilate in grapes of different botanical origin.

The grape is preeminently a North American plant, for among the number of species of the genus Vitis found in the world, which is generally considered by botanists to range from 40 to 60,[4] more than half are natives of this continent. The number of varieties of the grape described in American viticultural literature is said to be more than 2,000, while twice as many more are mentioned in European treatises on the vine.

Inasmuch as the principal object of the present investigation was to determine whether methyl anthranilate is a common constituent of the grape or whether its occurrence is restricted to certain distinct species of the genus Vitis, or the varieties derived therefrom by cultivation, consideration may first be given to the recognized botanical origin of the grapes examined, which are designated by their popular names.

A very complete elucidation of the subject of American grapes is contained in a work by U. P. Hedrick,[5] to whom we are indebted for much of the information concerning the botanical relationships of the numerous varieties of grapes used in the present investigation. Hedrick [6] has also published a smaller and more recent work on this subject.

In the classification of grapes a primary distinction is made between those of the Old World and those of the New World, and it has been noted that the grape is probably influenced to a greater degree by soil, climate, and culture than any other fruit. In the Old World a single species of

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[1] Accepted for publication Aug. 12, 1922.

[2] POWER, Frederick B., and CHESNUT, Victor K. THE OCCURRENCE OF METHYL ANTHRANILATE IN GRAPE JUICE. *In* Jour. Amer. Chem. Soc., v. 43, p. 1741–1742. 1921.

[3] POWER, Frederick B. THE DETECTION OF METHYL ANTHRANILATE IN FRUIT JUICES. *In* Jour. Amer. Chem. Soc., v. 43, p. 377–381. 1921. Bibliographical footnotes.

[4] In Index Kewensis, pt. 4 and sup. 1–5. Oxford. 1895, 1901–15, several hundred species of Vitis are enumerated, but, as stated by Hedrick,[5] the number depends upon the arbitrary limits set for a species and our knowledge of the genus is as yet too meager to set these limits with certainty.

[5] HEDRICK, U. P. THE GRAPES OF NEW YORK. xv, 564 p., 101 col. pl., 1 port. Albany. 1908. Bibliography and references, p. 531–536. (N. Y. Agr. Exp. Sta. Rpt. 1907, pt. 2.)

[6] HEDRICK, U. P. MANUAL OF AMERICAN GRAPE-GROWING. xiii, 458 p., 54 fig., 32 pl. 1919.

grape is cultivated—the *Vitis vinifera* L. and its varieties. This species is grown for the production of wine and for making raisins because its varieties have a higher sugar and solid content than those of the American species, which are cultivated chiefly for table use. The European varieties are considered to have a more delicate and richer vinous flavor and a more agreeable aroma, with less acidity, than American table grapes, although the latter are more refreshing and their unfermented juice makes a more pleasant drink.

The most commonly cultivated native American species of grape is the *Vitis labrusca* L., which, in the wild state, occurs in black-, white-, and red-fruited forms. Its numerous varieties possess a characteristic flavor and aroma, often described by the terms "foxy" or "musky," and if not too pronounced this musk-like flavor is often very agreeable. A typical representative of this species is the Concord grape. Another northern species, the varieties of which are esteemed for wine making, is *V. riparia* Michx. The variety under cultivation which is considered one of the best representatives of this species is the Clinton grape.

Many attempts have been made to grow the European grape in the eastern part of North America, but for various reasons these have resulted in failure, the climate particularly not being suitable.[7] It is only in the regions west of the Rocky Mountains, and more particularly in California, that the varieties of *Vitis vinifera* are successfully cultivated in America, and the great viticultural interests of the far West are said to be founded upon the success of this one species, with which the native grape can not compete for any purpose. Nevertheless, American species are regarded as indispensable in this western region for stocks upon which to graft the *vinifera* varieties, and it is said to be probable that the time is not far distant when all California vines will be growing upon roots of American species. The number of *vinifera* varieties grown for wine, raisins, and table grapes extends into hundreds, and it has been noted that the great success met with in the cultivation of this species west of the Great Continental Divide makes all the more remarkable the fact that in no place east of the Divide will varieties of it thrive.

One of the species of Vitis which for many years has been the favorite grape in many sections of the South is *V. rotundifolia* Michx. This is represented by the so-called Scuppernong or Muscadine grape. The fruit of all the varieties of this species is characterized by a strong musky aroma, but it is lacking in both sugar and acid. It is not suitable for table use, chiefly because the berries ripen unevenly and when ripe drop from the clusters. Another southern species is *V. aestivalis* Michx., best represented by the Norton grape.

I. GRAPES OF THE EASTERN STATES

The following varieties of the grape, which are cultivated in the eastern part of North America, were examined for methyl anthranilate. The juices from all these varieties were personally prepared by Dr. J. S. Caldwell from ripe fruit grown at Vineland, N. J., and were therefore perfectly authentic.[8] In this enumeration the accepted botanical derivation of each variety is noted, together with the general characters of the fruit.

[7] Further efforts in this direction have recently been made at the New York Agricultural Experiment Station with more promise of success.

[8] All these juices were cold-pressed and were pasteurized by heating at a temperature of 75 to 80° C. Samples of juice of the Worden and Concord grapes were also prepared by heating the crushed grapes to 80° before pressing. The results of the tests for methyl anthranilate with both cold- and hot-pressed juices were practically identical.

A. VARIETIES WHICH GAVE A STRONG REACTION FOR METHYL ANTHRANILATE

CONCORD (*V. labrusca*).—This is regarded as a pure-bred *labrusca*. The berries are black with a blue bloom. It is the most widely grown of the grapes of this continent, and furnishes 75 per cent or more of the grapes of eastern America. Grape juice is made almost entirely from this fruit, and since the European grape does not make a good unfermented juice the industry is subject to no competition from California or Europe.

EATON (*V. labrusca*).—This is considered to be a pure-bred seedling of the Concord variety. The berries are large and black and are covered with a heavy blue bloom.

WORDEN (*V. labrusca*).—An offspring of the Concord grape. The berries vary from dark purplish black to black and are covered with a heavy blue bloom.

NIAGARA (*labrusca-vinifera* hybrid).—One of the parents of this grape is the Concord. The berries are light green and are covered with a thin gray bloom.

MARTHA (possibly a *labrusca-vinifera* hybrid).—This is a seedling of the Concord, which it resembles greatly but from which it differs in several particulars. The berries are light green with a tinge of yellow and are covered with a thin gray bloom.

IVES (possibly a *labrusca-aestivalis* hybrid).—The berries are jet black and are covered with a moderate amount of blue bloom.

DELAWARE (*labrusca-bourquiniana-vinifera* hybrid).—The berries vary from small to medium, are light red, and are covered with a thin lilac bloom.

MONTEFIORE (*riparia-labrusca* hybrid).—The berries are black and glossy and are covered with an abundant blue bloom.

MISSOURI RIESLING (*riparia-labrusca* hybrid).—The berries are pale or yellowish green, changing to light red or having a tinge of pink when fully ripe, and are covered with a thin gray bloom.

B. VARIETIES WHICH GAVE A SLIGHT BUT DISTINCT REACTION FOR METHYL ANTHRANILATE

POCKLINGTON (*V. labrusca*).—A so-called white grape and seedling of the Concord. The berries are yellowish green and are covered with a thin gray bloom.

LUCILE (*V. labrusca*).—The berries are dark red and are covered with a thin lilac bloom.

VERGENNES (*V. labrusca*).—The berries are either light red or dark red and are covered with a lilac bloom.

BRIGHTON (*labrusca-vinifera* hybrid).—The berries are either light red or dark red and are covered with a dark lilac bloom.

CAMPBELL EARLY (*labrusca-vinifera* hybrid).—The berries are dark purplish black and are covered with a heavy blue bloom.

WOODRUFF RED (possibly a *labrusca-vinifera* hybrid).—This has been supposed to be a cross of the Catawba and Concord grape. The berries are dark brick red and are covered with a thin lilac to faint blue bloom.

LINDLEY (*labrusca-vinifera* hybrid).—The berries are dark brick red and are covered with a lilac or faint blue bloom.

HERBERT (*labrusca-vinifera* hybrid).—The berries are a rather dull black and are covered with a thick blue bloom.

DIAMOND (*labrusca-vinifera* hybrid).—The berries are green and are covered with a thin gray bloom.

MERRIMAC (*labrusca-vinifera* hybrid).—The berries are black and glossy and are covered with an abundant blue bloom.

AGAWAM (*labrusca-vinifera* hybrid).—The berries are dark and dull purplish red, somewhat resembling the Catawba, and are covered with a lilac bloom.

WILDER (*labrusca-vinifera* hybrid).—The berries are large, purplish black to black, and are covered with a heavy lilac bloom.

BARRY (*labrusca-vinifera* hybrid).—The berries are large, dark purplish black to black, and are covered with a heavy blue bloom.

PERKINS (*labrusca-vinifera* hybrid).—The berries vary from large to medium, are dull green, changing to pale lilac or light red when fully ripe, and are covered with a rather abundant gray or lilac bloom.

ISABELLA (*labrusca-vinifera* hybrid).—The berries vary from medium to large, are deep black, and are covered with considerable blue bloom.

EARLY VICTOR (possibly a *labrusca-bourquiniana* hybrid).—The berries are dark purplish black and are covered with a heavy blue bloom.

BRILLIANT (*labrusca-vinifera-bourquiniana* hybrid).—The berries are dark red, rather glossy, and are covered with an abundant lilac bloom.

DIANA (possibly a *labrusca-vinifera-aestivalis* hybrid).—This is a seedling of the Catawba to which it bears a strong resemblance. The berries are somewhat irregular in size, rather light red, and are covered with a thin lilac bloom.

EUMELAN (*labrusca-vinifera-aestivalis* hybrid).—The berries are black and glossy and are covered with an abundant lilac bloom.

CLEVENER (possibly a *labrusca-riparia-aestivalis* hybrid).—The berries are black and rather glossy and are covered with a blue bloom.

ROMMEL (*labrusca-riparia-vinifera* hybrid).—The berries are light green with a yellow tinge, glossy, and are covered with a moderate amount of gray bloom.

ELVIRA (*riparia-labrusca* hybrid).—The berries are greenish, with a yellowish tinge, and are covered with a fair amount of gray bloom.

CLINTON (*riparia-labrusca* hybrid).—The berries are dark purplish black to black, glossy, and are covered with a rather thick blue bloom.

IRONCLAD OR DIOGENES (*riparia-labrusca* hybrid).—The berries are small, jet black, glossy, and are covered with blue bloom.

FRANKLIN (*riparia-labrusca* hybrid).—A minor variety, which is said to resemble the Clinton very closely.

CYNTHIANA (*aestivalis-labrusca* hybrid).—The berries are small and black and are covered with a moderate amount of blue bloom.

NORTON (*aestivalis-labrusca* hybrid).—The berries are small and black, somewhat glossy, and are covered with a heavy blue bloom.

GOETHE (*vinifera-labrusca* hybrid).—This resembles to a marked degree the White Malaga grape of European fame. The berries are large, pale red, and are covered with a thin gray or slightly lilac bloom.

DUTCHESS (*vinifera-labrusca* hybrid, with possibly *bourquiniana* and *aestivalis*).—The berries are pale yellowish green and are covered with a thin gray bloom.

KING PHILIP (a minor variety, considered to be a *vinifera-labrusca-riparia* hybrid).—The berries are purple.

C. VARIETIES WHICH GAVE NO REACTION FOR METHYL ANTHRANILATE

CATAWBA (*labrusca-vinifera* hybrid).—The berries are intermediate in size, dull purplish red, and are covered with a moderate amount of lilac bloom.

ULSTER (*labrusca-vinifera* hybrid).—The parents of this grape are said to be the Catawba pollinated by a wild *aestivalis*. The berries are dark dull red and are covered with a thin, light to dark lilac bloom.

SALEM (*labrusca-vinifera* hybrid).—The berries vary from large to medium, are very dark dull red, and are covered with a medium amount of blue bloom.

HERBEMONT (*V. bourquiniana*).—The berries are reddish black or brown, with an abundant blue bloom.

NECTAR (*labrusca-bourquiniana* hybrid, with possibly *vinifera*).—The berries are intermediate in size, dark purplish black, and are covered with a heavy blue bloom.

NOAH (*riparia-labrusca* hybrid).—The berries are small, light green tinged with yellow, and are covered with a thin gray bloom.

CANADA (*riparia-labrusca-vinifera* hybrid).—The berries vary from medium to small, are purplish black to black, glossy, and are covered with a heavy dark blue bloom.

BERCKMANS (*riparia-labrusca-bourquiniana* hybrid).—The berries are intermediate in size, resemble the Delaware in color, and are covered with a lilac bloom.

In connection with the preceding classification, which is based upon the occurrence or absence of methyl anthranilate, it has been deemed of interest to present a survey of the numerous varieties of the grape examined by arranging them according to their botanical derivation. The variations respecting the occurrence of methyl anthranilate in the different botanical groups may thus readily be seen, and the relative amounts and the absence of the particular ester are indicated by the following signs: ++ indicates a strong reaction; + indicates a slight but distinct reaction; − indicates a negative result.

Labrusca.

++ Concord.
++ Worden.
+ Pocklington.
++ Eaton.
+ Lucile.
+ Vergennes.

Labrusca-vinifera.

++ Niagara.
+ Brighton.
+ Woodruff Red.
+ Herbert.
+ Merrimac.
+ Wilder.
+ Perkins.
– Catawba.
++ Martha.
+ Campbell Early.
+ Lindley.
+ Diamond.
+ Agawam.
+ Barry.
+ Isabella.
– Salem.
– Ulster.

Labrusca-bourquiniana.

+ Early Victor.

Labrusca-aestivalis.

++ Ives.

Labrusca-vinifera-bourquiniana.

+ Brilliant.

Labrusca-vinifera-aestivalis.

+ Diana.
+ Eumelan.

Labrusca-bourquiniana-vinifera.

++ Delaware.
– Nectar.

Labrusca-riparia-vinifera.

+ Rommel.

Labrusca-riparia-aestivalis.

+ Clevener.

Vinifera-labrusca.

+ Goethe.
+ Dutchess.

Vinifera-labrusca-riparia.

+ King Philip.

Riparia-labrusca.

++ Montefiore.
+ Clinton.
+ Franklin.
++ Missouri Riesling.
+ Elvira.
+ Ironclad (Diogenes).
– Noah.

Riparia-labrusca-vinifera.

– Canada.

Riparia-labrusca-bourquiniana.

– Berckmans.

Aestivalis-labrusca.

+ Cynthiana.
+ Norton.

Bourquiniana.

– Herbemont.

II. GRAPES OF THE SOUTHERN STATES

The grapes of the Southern States, as previously noted, are derived chiefly from the species known as *Vitis rotundifolia* or its varieties. Through the courtesy of Dr. J. S. Caldwell we were provided with samples of so-called Muscadine grape juice which had been prepared by Charles Dearing and Guy F. Yerkes, of Willard, N. C. These samples were designated by the following popular names of the particular varieties of grape: (1) Thomas, (2) Scuppernong, (3) Latham, (4) Mish. All the juices were cold-pressed and were clear, pale yellow or brownish yellow liquids. They had a sweetish, honeylike odor, quite unlike that of the northern grape juices. The test for methyl anthranilate, which was conducted with 500 cc. of the respective samples, gave in all cases a perfectly negative result.

III. CALIFORNIA GRAPES

The grapes of California may be considered as representative of the European species, *Vitis vinifera*, of which a very large number of varieties have been produced through cultivation. Two samples of juice from grapes of the 1921 crop were kindly supplied by Prof. W. V. Cruess,

of the University of California, and reported by him to be true to name. These were designated as (1) Muscat and (2) Petite Sirah. The Muscat juice was expressed without heating and was pasteurized in the container at 175° F. The Petite Sirah juice was prepared by heating the crushed grapes to about 160° F., and pasteurizing in the container at 175° F. The Muscat juice was a pale yellow liquid, having a sweetish odor. When distilled for the purpose of the test, the first portion of the distillate was observed to be very fragrant, the odor being somewhat like that of nutmeg. The Petite Sirah juice was a deep purplish red liquid, without much odor but having a pleasant, very sweet taste. No reaction for methyl anthranilate could be obtained with either of these liquids when portions of 500 cc. were subjected to the special test.

We are indebted to Mr. Elmer Snyder, of Fresno, Calif., for several additional samples of California juices, which were supplied by the California Wine Association and designated as follows: (1) Muscat, (2) Burger, (3) Sauvignon vert, (4) Zinfandel, (5) Alicante Bouschet. The first three of these samples were pale yellow or brownish yellow liquids, while the fourth and fifth had a deep purplish red color and a somewhat distinctive odor. The light-colored juices were cold-pressed, while the purplish red ones were obtained by heating the grapes before pressing. In none of these five samples of grape juice could any trace of methyl anthranilate be detected.

COMMERCIAL GRAPE JUICES

In connection with the examination of juices from definite varieties of the grape it was deemed desirable to ascertain the character of some of the commercial products, of which a considerable number were available.

The products from 13 manufacturers of grape juice in the Eastern or Central States, all of which were either definitely declared to have been made from Concord grapes or were deep red liquids having the characters of pure Concord juices, gave evidence of the presence of methyl anthranilate, but not in larger proportions than had been found in perfectly authentic juices. The same positive results were obtained from a red juice produced in the State of Washington. This juice was stated to have been made from Concord and Worden grapes, and it was collected directly from the press by an official inspector from the Bureau of Chemistry, United States Department of Agriculture. On the other hand, one sample of a California product, although labeled "Concord Grape Juice" and containing methyl anthranilate, was a yellowish liquid having a sweetish, honeylike odor, quite unlike that of a pure Concord juice. Its authenticity and purity were therefore extremely doubtful. Very slight reactions for methyl anthranilate were obtained with two red juices, produced by Eastern manufacturers, which were not designated as Concord; and traces of the ester were indicated in two commercial Catawba juices, both of which were clear, pale yellow liquids containing sulphur dioxid.

Only a few commercial juices gave results which indicated the complete absence of methyl anthranilate. These comprised the following products: (1) Two samples designated simply as grape juice, which were clear, pale yellow liquids; (2) one sample specifically designated as Catawba juice, which was a clear, bright yellow liquid; (3) three samples of red California juices, one of which was stated to have been prepared from ripe wine

grapes of the transplanted European varieties, while another was designated as Alicante grape juice, and the third sample was only claimed to be the juice from ripe California grapes; (4) one sample of red juice obtained from the State of Washington and presumably prepared from a variety of the European grape; the expression of this juice was supervised by an inspector from the Bureau of Chemistry, United States Department of Agriculture.

In the course of the preceding investigation a sample of loganberry juice was examined for the presence of methyl anthranilate but, as was anticipated, with a perfectly negative result.

CONCLUSIONS

In considering the results of this investigation it is of interest to observe that those grapes which are regarded by viticulturists as representing pure-bred *Vitis labrusca* contain methyl anthranilate. With few exceptions it was also found in varying amounts in all those varieties which represent hybrids of *V. labrusca*, and especially when this species predominates. On the other hand, this ester could not be detected in juices from California grapes, which are derived from the European *V. vinifera*, nor in juices from southern grapes, derived from *V. rotundifolia*. A negative result was likewise obtained with the one available sample of juice from the species known as *V. bourquiniana* Munson.

Inasmuch as considerable uncertainty appears to exist with regard to the botanical origin of several varieties of the grape it is possible that a determination of the presence or absence of methyl anthranilate may possess some diagnostic value. The extent to which this compound occurs in any particular variety may, however, be influenced in some degree by the soil and climate, as it doubtless would be by varying conditions of ripeness of the fruit.

Although it may be presumed that methyl anthranilate imparts a distinctive odor to those varieties of the grape in which it occurs, it evidently does not completely represent their odorous constituents. As already noted, it was not found in the juice of the Catawba grape, which possesses a characteristic aroma, and it has been observed that the botanical characters of this variety are those of *Vitis vinifera* crossed with *V. labrusca*. A complete chemical examination of the odorous constituents of grapes, which probably differ to some extent in the numerous varieties, still remains to be accomplished.

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EFFECT OF VARIOUS INORGANIC NITROGEN COMPOUNDS, APPLIED AT DIFFERENT STAGES OF GROWTH, ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT [1]

By JEHIEL DAVIDSON, *Soil Chemist*, and J. A. LECLERC, *formerly Chemist in Charge, Plant Chemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

In a series of experiments reported in 1917 and 1918 [2] sodium nitrate was applied at different stages of growth. When the sodium nitrate was applied at the first stage, when the crop was about 2 inches high, an increase in yield was obtained. When applied at the second stage, at the time of heading, the yield was not affected, but a higher nitrogen content, both in the grain and straw, was obtained, and yellow-berry was practically eliminated. When applied at the third or milk stage, almost no effect was observed. The wheat used in those experiments was a soft winter wheat. The terms flinty and yellow-berry refer not to the typical qualities of wheat designated by them but only to the color of the grain.[3]

The experiment discussed in this article was undertaken with two objects in view. One was to repeat the previous experiments with a hard winter wheat in a region where the prevalence of typical yellow-berry fluctuates from year to year; the other was to determine the effect of nitrogen derived from carriers other than sodium nitrate.

The experiment was carried out on the farm of the Claassen brothers, near Beatrice, Cage County, in the State of Nebraska,[4] in the summer of 1917.

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[1] Accepted for publication July 2, 1921.

[2] DAVIDSON, J., and LECLERC, J. A. THE EFFECT OF SODIUM NITRATE APPLIED AT DIFFERENT STAGES OF GROWTH ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT. *In* Jour. Amer. Soc. Agron., v. 9, no. 4, p. 145–154, 1917; v. 10, no. 5, p. 193–198, 1918.

[3] Since this work was completed, W. F. Gericke (ON THE PROTEIN CONTENT OF WHEAT. *In* Science, N. S., v. 52, p. 446–447. 1920; DIFFERENCES EFFECTED IN THE PROTEIN CONTENT OF GRAIN BY APPLICATIONS OF NITROGEN MADE AT DIFFERENT GROWING PERIODS OF PLANTS. *In* Soil Science, v. 14, p. 103–109. 1922) reported results which, with due allowance for the greenhouse conditions under which his experiments were carried out, are identical in their essential tendencies with the results just referred to. The two papers by Davidson and LeClerc,[2] which had been presented at consecutive annual meetings of the American Society of Agronomy before they were published, seem to have escaped Mr. Gericke's notice, as he asserts (Science, loc. cit.) that his results obtained with wheat, one of the plants studied, throw new light upon this protein question.

[4] The authors wish to acknowledge the valuable assistance of County Agent L. B. Rist and of the owners of the farm.

EXPERIMENTAL WORK

PLAN OF THE EXPERIMENT

The plan of the experiment is given in the following table:

Plot No.	Stage 1 (crop about 8 inches high).	Stage 2 (time of heading).	Stage 3 (milk stage).
1	Sodium nitrate (2 pounds).		
2	.....	Sodium nitrate (2 pounds).	
3	.....	.....	Sodium nitrate (2 pounds).
4	Potassium chlorid (1.8 pounds).		
5	.....	Potassium chlorid (1.8 pounds).	
6	.....	.....	Potassium chlorid (1.8 pounds).
7	Calcium nitrate (2 pounds).		
8	.....	Calcium nitrate (2 pounds).	
9	.....	.....	Calcium nitrate (2 pounds).
10	Ammonium sulphate (1.5 pounds).		
11	.....	Ammonium sulphate (1.5 pounds).	
12	.....	.....	Ammonium sulphate (1.5 pounds).
13	Sodium hydroxid (0.9 pound).		
14	.....	Sodium hydroxid (0.9 pound).	
15	.....	.....	Sodium hydroxid (0.9 pound).
16	Potassium nitrate (2.4 pounds).		
17	.....	Potassium nitrate (2.4 pounds).	
18	.....	.....	Potassium nitrate (2.4 pounds).
19	Sulphuric acid (298 cc.).		
20	.....	Sulphuric acid (298 cc.).	
21	.....	.....	Sulphuric acid (298 cc.).
22	Ammonium chlorid (1.3 pounds).		
23	.....	Ammonium chlorid (1.3 pounds).	
24	.....	.....	Ammonium chlorid (1.3 pounds).
25	Potassium hydroxid (1.3 pounds).		
26	.....	Potassium hydroxid (1.3 pounds).	
27	.....	.....	Potassium hydroxid (1.3 pounds).
28	Ammonium nitrate (0.94 pound).		
29	.....	Ammonium nitrate (0.94 pound).	

Plot No.	Stage 1 (crop about 8 inches high).	Stage 2 (time of heading).	Stage 3 (milk stage).
30	.....	.....	Ammonium nitrate (0.94 pound).
31	Water (25 gallons).....	Water (25 gallons).....	Water (25 gallons).
32	.....	Control.	
33	Sodium chlorid (1.4 pounds).		
34	.....	Sodium chlorid (1.4 pounds).	
35	.....	.....	Sodium chlorid (1.4 pounds).
36	Calcium chlorid (1.3 pounds).		
37	.....	Calcium chlorid (1.3 pounds).	
38	.....	.....	Calcium chlorid (1.3 pounds).
39	Hydrochloric acid (887 cc.).		
40	.....	Hydrochloric acid (887 cc.).	
41	.....	.....	Hydrochloric acid (887 cc.).
42	Potassium sulphate (2 pounds).		
43	.....	Potassium sulphate (2 pounds).	
44	.....	.....	Potassium sulphate (2 pounds).
45	Magnesium nitrate (1.7 pounds).		
46	.....	Magnesium nitrate (1.7 pounds).	
47	.....	.....	Magnesium nitrate (1.7 pounds).
48	Magnesium chlorid (1.1 pounds).		
49	.....	Magnesium chlorid (1.1 pounds).	
50	.....	.....	Magnesium chlorid (1.1 pounds).
51	Nitric acid (678 cc.).		
52	.....	Nitric acid (678 cc.).	
53	.....	.....	Nitric acid (678 cc.).
54	.....	Ammonia water(1,490 cc.).	
55	.....	.....	Ammonia water (1,490 cc.)
56	Water (25 gallons).....	Water (25 gallons).....	Water (25 gallons).
57	.....	Control.	

The applications of the various chemicals were calculated at the rate of 320 pounds of sodium nitrate to the acre. The nitrogen carriers were used in such proportions as to give an amount of nitrogen to the acre equal to that supplied by the application of sodium nitrate. The other elements were applied in such proportions as to give amounts to the acre equal to those supplied by the nitrogen carriers. The acids were used in amounts which were chemical equivalents of their respective neutral salts. The exact quantities of the chemicals used to the plot are shown in the foregoing table. The chemicals were applied in solution only, and 25 gallons of water were used in each case. The experiment was conducted in duplicate (series A and series B). An average of the results

of the two series was not taken, as the purpose of the work was to demonstrate tendencies rather than to obtain absolute figures.

The plots comprised 1 square rod each, with 2-foot alleys between them.

PRINCIPLES UNDERLYING SELECTION OF CHEMICALS

When flinty wheat kernels were soaked in the laboratory in a slightly acid solution they lost their brown color, and when yellow-berried kernels were soaked in a slightly alkaline solution they acquired the brown coloration characteristic of flinty grain. This suggested the idea that the color characteristics of flinty and yellow-berried grain constitute indicator reactions with alkalies and acids, as well as the possibility that the elimination of yellow-berry caused by the application of sodium nitrate was due to the residual effect of the sodium base.

The nitrogen carriers used in this experiment were accordingly of two distinct groups: Nitric acid and its salts and ammonia and its salts. The salts of the nitric acid belong to the physiologically alkaline group. Since the plants use the nitrogen of the acid radicles more readily than that of the basic elements, residues of basic radicles are left. Following the same reasoning, the salts of ammonia may be placed in the physiologically acid group. Ammonium nitrate is a physiologically neutral salt. Nitric acid would have the status of a nitrate, since it attacks the physiologically neutral salts of the soil, particularly the carbonates, thus being transformed into a physiologically alkaline substance. Ammonia, which nitrifies very readily, would have approximately the same status as nitric acid. The status of potassium nitrate would depend upon the rate of assimilation of potassium and nitrogen by the plants, which would vary with soil conditions. If these two elements were assimilated by the plants at the same rate, the status of potassium nitrate would be that of a physiologically neutral substance. Should these two elements be assimilated at different rates, its status would always be that of a physiologically alkaline substance. Should nitrogen be assimilated more readily, a residue of basic potassium would be the result. On the other hand, should potassium be assimilated more readily, the residue would be nitric acid which, as just explained, would act as a physiologically alkaline substance.

The same principle underlies the selection of the other chemicals. It was expected that the acids and alkalies would produce direct effects of alkalinity and acidity. The salts of potassium were counted on to produce physiological acidity. The physiologically neutral salts were intended to serve as controls with reference to the residual acid and basic radicles of the nitrogen carriers.

The term "residual effect" is used in this connection to signify the possible effect of residual acid or basic radicles on the soil reaction, as well as their effect on the physiological reaction of the plant tissues.

FACTORS WHICH TENDED TO DISTURB THE REGULARITY OF RESULTS

The plots were laid out in the spring just before the first application. Due to the fact that the wheat in Nebraska in that year suffered heavily from late frosts, it was impossible to obtain uniform plots. The part of the field used for series A was fairly uniform, and the plots were laid out consecutively. The section of the field which was available for series B was entirely lacking in uniformity. An attempt was made to select fairly

uniform plots for the three applications of each chemical and also for certain groups of chemicals. The more uniform plots were assigned to the nitrogen carriers. Consequently, the control plots were not true representatives of normal conditions. Deviations from the controls were not necessarily due to the treatments in the case of the tendencies which were not sharply defined but which might have been detected under normal conditions.

Another element of disturbance affecting the regularity of the results was introduced by the fact that there was no uniform transition from one stage to another. When most of the plants on the plots were headed out some had not yet reached that stage. The same thing occurred during the transition from the second to the third stage.

Moreover, a heavy rainfall, coming just as the treatment of one series was finished, interfered with the second stage application. Part of the chemicals applied to that series were thus probably washed out. The other series had to be treated when the soil was completely saturated. As a result, a portion of the chemicals which were applied in solution ran off from some of the plots. This probably accounts for part of the irregularities which will be found in the tables.

With all these disturbing factors, however, the principal tendencies resulting from the application of the various nitrogen carriers were not obscured, due probably to the fact that of the 114 plots of the experiment 54 received nitrogen. The remaining 60 plots served as controls with reference to the effects of this element. Similarly, the plots which received the nitrogen in the third stage frequently served as controls for the plots of the other two stages of the groups.

TABLE I.—*Total yield and percentage of grain from plots to which chemicals were applied at various stages of growth*

Chemicals applied.	Series A.						Series B.					
	Total yield.			Percentage of grain.			Total yield.			Percentage of grain.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
	Lbs.	*Lbs.*	*Lbs.*				*Lbs.*	*Lbs.*	*Lbs.*			
Sodium nitrate	50.2	31.1	28.5	36.9	41.9	42.7	34.7	26.5	23.4	33.3	42.3	38.2
Potassium chlorid	30.0	33.2	24.2	42.9	40.5	40.4	26.0	25.5	23.7	39.6	41.9	41.4
Calcium nitrate	45.9	33.0	23.1	37.3	41.5	42.8	33.5	23.0	24.8	35.9	38.2	38.3
Ammonium sulphate	39.5	33.5	29.3	34.5	39.3	38.6	29.5	25.4	22.5	40.1	39.4	36.8
Sodium hydroxid	27.0	25.0	21.7	36.8	45.8	40.4	27.2	28.2	21.5	42.4	39.8	42.4
Potassium nitrate	39.8	29.5	27.5	38.5	39.4	40.7	33.5	31.8	25.4	34.8	40.2	39.5
Sulphuric acid	26.0	21.5	24.6	42.2	41.3	39.3	22.8	24.2	25.2	41.8	42.6	38.8
Ammonium chlorid	39.0	32.0	23.7	46.3	41.3	42.6	30.6	26.7	21.0	39.5	42.3	43.0
Potassium hydroxid	22.0	23.3	29.9	44.1	41.8	42.6	25.0	24.8	23.0	42.6	41.7	42.7
Ammonium nitrate	49.7	37.9	29.7	38.2	42.1	43.2	38.2	26.3	25.6	34.3	40.8	42.7
Water		24.5			43.6			23.5			44.3	
Control		24.7			43.8			24.5			41.8	
Sodium chlorid	24.2	27.5	30.0	44.0	43.1	44.1	24.7	23.8	22.9	44.2	41.9	42.5
Calcium chlorid	33.8	33.5	31.5	42.0	44.5	37.4	21.8	23.0	24.0	44.6	43.3	41.9
Hydrochloric acid	25.1	23.4	25.0	41.6	40.9	42.9	25.2	23.2	23.5	42.7	42.8	43.7
Potassium sulphate	32.7	32.1	30.7	41.4	41.5	41.9	27.5	25.5	23.0	42.7	42.3	41.6
Magnesium nitrate	34.4	26.1	23.6	40.3	42.1	45.4	33.9	26.4	21.3	37.2	41.4	42.6
Magnesium chlorid	34.7	31.2	27.1	41.4	43.2	43.8	27.0	25.0	24.4	43.8	43.5	38.2
Nitric acid	40.2	27.2	25.9	33.9	38.2	38.6	35.0	26.1	24.3	33.9	37.7	40.2
Ammonia					42.2	36.0					42.3	42.5
Water		28.5			37.7			21.5			38.9	
Control		29.6			40.7			21.7			39.8	

TABLE II.—*Percentage of yellow berry in wheat from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.			Series B.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	10.0	1.9	19.4	7.2	5.1	17.7
Potassium chlorid	25.7	24.3	41.3	21.5	26.2	20.0
Calcium nitrate	13.5	3.2	15.0	9.7	5.2	22.6
Ammonium sulphate	15.5	3.0	33.6	5.7	1.8	15.3
Sodium hydroxid	21.4	21.4	21.8	15.9	23.4	13.9
Potassium nitrate	14.5	2.7	12.6	4.9	2.2	15.1
Sulphuric acid	32.3	28.8	42.7	21.9	29.1	14.7
Ammonium chlorid	25.8	4.5	31.7	10.6	4.4	14.3
Potassium hydroxid	26.8	24.3	20.8	9.9	8.3	17.6
Ammonium nitrate	12.4	3.1	11.7	4.1	3.1	4.8
Water		24.4			16.5	
Control		22.2			10.2	
Sodium chlorid	31.2	30.2	34.6	18.5	9.9	15.0
Calcium chlorid	31.5	31.5	24.0	12.1	12.0	12.6
Hydrochloric acid	19.2	26.1	32.4	8.1	5.5	10.7
Potassium sulphate	33.2	31.1	24.8	13.3	15.8	15.2
Magnesium nitrate	25.3	9.5	22.1	10.3	5.6	13.7
Magnesium chlorid	34.8	28.4	33.0	11.3	14.4	20.1
Nitric acid	12.1	4.2	14.7	3.7	1.8	8.9
Ammonia	.....	1.0	15.3	.....	0.3	12.9
Water		34.1			17.0	
Control		25.0			8.5	

TABLE III.—*Weight per bushel and weight per 1,000 kernels of wheat from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Weight per bushel.			Weight per 1,000 kernels.			Weight per bushel.			Weight per 1,000 kernels.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
	Lbs.	*Lbs.*	*Lbs.*	*Gm.*	*Gm.*	*Gm.*	*Lbs.*	*Lbs.*	*Lbs.*	*Gm.*	*Gm.*	*Gm.*
Sodium nitrate	59.8	60.1	60.5	26.8	28.8	30.0	59.1	60.5	60.5	25.8	28.7	28.6
Potassium chlorid	60.1	60.4	60.5	29.2	29.7	31.9	60.8	61.2	61.2	28.1	28.9	29.1
Calcium nitrate	59.0	59.8	61.1	27.3	27.9	30.0	60.5	60.5	61.5	26.4	27.9	30.6
Ammonium sulphate	60.1	60.5	59.8	26.6	28.1	29.7	60.5	60.8	60.5	28.0	28.3	30.5
Sodium hydroxid	60.8	61.5	60.8	28.4	28.7	28.2	60.8	60.8	61.2	29.6	29.2	28.2
Potassium nitrate	60.5	60.5	60.8	28.6	29.1	29.2	60.5	60.5	61.9	27.0	27.5	28.1
Sulphuric acid	61.1	61.1	60.5	28.6	28.6	27.5	61.2	61.2	60.2	28.5	28.6	28.0
Ammonium chlorid	60.6	61.1	61.1	28.3	28.3	27.7	60.2	58.6	61.9	28.2	27.4	27.6
Potassium hydroxid	61.5	61.9	61.1	28.2	28.3	29.0	61.9	61.2	61.2	29.1	28.9	28.0
Ammonium nitrate	60.8	61.1	62.6	27.5	28.5	30.7	60.5	60.5	61.2	27.6	26.4	28.7
Water		61.1			28.3			61.9			29.7	
Control		61.5			27.3			61.2			27.6	
Sodium chlorid	60.8	60.8	61.1	27.1	27.1	27.8	61.2	61.2	61.9	29.1	28.6	28.4
Calcium chlorid	61.9	61.9	61.1	28.9	29.3	28.6	61.9	63.2	61.5	28.5	29.7	29.5
Hydrochloric acid	61.1	61.9	61.9	27.5	29.7	27.2	61.5	61.9	60.5	29.2	30.1	29.1
Potassium sulphate	61.9	61.5	61.9	29.5	27.3	29.3	61.2	61.9	61.5	29.3	29.2	29.2
Magnesium nitrate	61.1	61.9	61.9	27.7	28.6	28.1	60.5	60.8	61.9	28.5	28.2	28.1
Magnesium chlorid	61.5	61.9	60.8	29.4	29.3	29.0	61.5	61.2	62.9	28.7	29.5	30.1
Nitric acid	60.5	61.5	61.5	25.9	27.1	27.8	60.5	60.8	61.9	25.9	28.7	30.1
Ammonia	.....	61.1	61.9	.....	30.1	30.1	.....	60.8	62.2	.....	29.3	30.5
Water		60.5			28.7			61.5			28.7	
Control		61.4			28.0			61.2			28.5	

TABLE IV.—*Correlation between protein content and weight per bushel and weight per 1,000 kernels of wheat*

Place of growth.	Variety.	Year.	Protein content.	Weight per 1,000 kernels.	Weight per bushel.
			Per cent.	*Gm.*	*Pounds.*
Kansas	Crimean	1907	22.2	21.3	51.3
Do	do	1907	22.2	20.5	51.3
Do	do	1908	14.8	28.4	58.1
Do	do	1908	14.7	28.8	58.2
California	Kubanka	1907	9.1	39.1	61.5
Do	do	1907	9.9	41.4	62.0
Do	do	1908	15.2	36.3	59.4
Do	do	1908	14.7	44.8	61.2
South Dakota	do	1907	13.9	37.9	63.0
Do	do	1907	12.9	39.4	63.7
Do	do	1908	16.6	24.9	57.8
Do	do	1908	16.5	29.8	60.2

TABLE V.—*Percentage of protein and phosphoric acid in grain from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Protein (N×5.7).			Phosphoric acid (P_2O_5).			Protein (N×5.7).			Phosphoric acid (P_2O_5).		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	14.1	14.7	15.3	1.04	1.03	1.18	15.9	16.6	14.8	0.84	0.99	1.03
Potassium chlorid	13.2	13.1	12.6	1.15	1.24	1.02	13.6	13.2	13.3	1.16	1.14	1.15
Calcium nitrate	.....	14.4	14.2	1.00	1.10	1.15	14.5	15.9	14.3	.92	1.03	1.07
Ammonium sulphate	13.5	14.5	12.8	1.00	1.05	1.14	14.4	15.6	14.0	.81	.98	1.07
Sodium hydroxid	12.3	12.4	12.8	1.13	1.05	1.18	13.4	13.3	13.1	1.17	1.10	1.14
Potassium nitrate	13.2	14.7	14.5	1.02	.98	1.12	15.0	16.9	13.5	.87	1.11	1.13
Sulphuric acid	12.4	12.5	12.3	1.12	1.14	1.21	13.2	13.2	12.9	1.13	1.14	1.16
Ammonium chlorid	12.4	13.9	13.0	.98	1.04	1.16	14.0	14.4	13.7	1.05	1.07	1.12
Potassium hydroxid	12.4	12.7	13.2	1.20	1.18	1.18	13.3	13.3	13.0	1.14	1.19	1.13
Ammonium nitrate	13.8	13.2	14.8	1.15	1.09	1.15	13.8	16.6	14.0	.99	1.06	1.17
Water		12.2			1.17			13.2			1.13	
Control		12.5			1.18			13.3			1.19	
Sodium chlorid	12.9	13.0	12.5	1.14	1.20	1.20	13.3	13.3	13.3	1.16	1.13	1.15
Calcium chlorid	12.5	12.7	12.9	1.16	1.19	1.18	13.4	13.7	13.2	1.14	1.16	1.17
Hydrochloric acid	13.0	12.8	12.5	1.18	1.18	1.15	13.5	13.3	13.2	1.15	1.13	1.20
Potassium sulphate	12.5	12.4	13.0	1.15	1.19	1.21	13.8	13.0	13.5	1.15	1.09	1.16
Magnesium nitrate	13.0	14.2	14.1	1.04	1.03	1.17	13.3	14.4	13.9	1.05	.99	1.08
Magnesium chlorid	12.9	13.2	12.9	1.15	1.18	1.14	13.8	13.6	13.2	1.08	1.12	1.11
Nitric acid	13.7	15.4	14.6	.98	.98	1.06	15.2	16.7	14.4	.94	.97	1.10
Ammonia	.....	15.9	13.7	.....	1.20	1.20	.....	16.3	14.3	.....	1.07	1.06
Water		12.5			1.11			12.9			1.11	
Control		12.6			1.10			13.6			1.12	

TABLE VI.—*Percentage of protein and phosphoric acid and straw from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Protein (N×6.25).			Phosphoric acid (P_2O_5).			Protein (N×6.25).			Phosphoric acid (P_2O_5).		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	3.27	3.45	2.84	0.18	0.17	0.19	3.98	4.06	3.74	0.12	0.15	0.17
Potassium chlorid	2.51	2.84	2.49	.23	.24	.19	3.45	3.26	3.04	.24	.25	.24
Calcium nitrate	2.91	3.78	3.09	.15	.20	.25	3.99	4.41	3.32	.16	.20	.21
Ammonium sulphate	3.58	3.91	3.26	.....	.....	.....	3.12	3.59	3.73	.12	.15	.24
Sodium hydroxid	3.54	2.92	3.35	.29	.26	.27	3.12	2.77	2.77	.24	.21	.20
Potassium nitrate	3.06	4.20	3.51	.20	.21	.21	3.90	4.07	3.05	.18	.19	.20
Sulphuric acid	2.72	3.06	3.21	.23	.26	.32	3.34	2.64	3.05	.25	.19	.19
Ammonium chlorid	3.39	3.61	2.93	.20	.20	.23	3.60	3.59	3.26	.18	.18	.20
Potassium hydroxid	2.72	2.51	2.66	.23	.22	.23	2.97	3.25	3.25	.22	.22	.21
Ammonium nitrate	2.92	3.65	3.99	.17	.19	.19	3.53	4.43	3.33	.16	.20	.21
Water		2.50			.19			2.51			.14	
Control		2.71			.21			2.63			.22	
Sodium chlorid	2.42	2.62	2.68	.21	.20	.21	2.68	2.85	2.86	.23	.21	.22
Calcium chlorid	2.49	2.01	2.48	.19	.21	.21	3.04	2.76	2.63	.24	.18	.20
Hydrochloric acid	2.89	2.49	2.75	.21	.21	.19	2.97	2.86	3.04	.23	.18	.26
Potassium sulphate	2.63	2.83	2.49	.20	.25	.26	2.63	2.64	2.86	.16	.19	.25
Magnesium nitrate	2.83	3.31	2.81	.16	.17	.18	3.12	3.45	2.84	.19	.16	.17
Magnesium chlorid	2.48	2.47	2.55	.21	.22	.20	2.65	3.03	3.32	.16	.20	.24
Nitric acid	3.02	4.55	3.36	.15	.20	.21	3.60	3.47	3.25	.17	.20	.21
Ammonia	.....	3.99	3.03	.....	.20	.22	.....	.....	.....	.....	.18	.23
Water		2.82			.24		.....	.....	.....		.23	
Control		2.83			.22		.....	.....	.....		.25	

TABLE VII.—*Lack of correlation between nitrogen and phosphoric acid content of wheat grown in Arlington on plots to which various chemicals were applied*

Chemicals applied.	Stage of growth.	Protein (N×5.7).	Phosphoric acid (P_2O_5).	Chemicals applied.	Stage of growth.	Protein (N×5.7).	Phosphoric acid (P_2O_5).
		Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
Sodium nitrate	2	16.4	1.02	Magnesium sulphate	3	11.3	0.98
Potassium nitrate	1	17.0	1.01	Potassium chlorid	2	11.45	1.03
Ammonium chlorid	1	17.1	1.00	Do	3	10.9	1.00
Ammonium nitrate	1	16.4	1.03	Calcium chlorid	3	11.7	1.01
Do	2	16.9	1.02	Potassium sulphate	1	11.2	1.02

TABLE VIII.—*Percentage of ash and silica in straw from plots to which chemicals were applied at three stages of growth*

Chemicals applied.	Series A.						Series B.					
	Ash.			Silica.			Ash.			Silica.		
	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.	Stage 1.	Stage 2.	Stage 3.
Sodium nitrate	11.0	11.3	14.0	8.1	8.0	10.8	11.2	11.9	12.2	6.1	7.3	8.6
Potassium chlorid	13.1	12.0	11.7	10.7	9.0	9.3	12.2	12.0	11.9	8.8	8.8	8.6
Calcium nitrate	10.0	11.8	12.8	5.8	7.3	9.0	11.0	11.1	11.7	6.7	6.5	8.4
Ammonium sulphate	9.42	9.60	11.3	5.5	5.6	8.0	11.1	11.2	11.5	7.2	7.0	8.3
Sodium hydroxid	12.5	12.8	13.2	8.9	9.6	10.2	14.3	12.5	12.8	10.5	8.9	9.3
Potassium nitrate	11.1	11.8	11.8	8.1	7.9	8.3	11.9	12.3	11.9	8.1	7.2	8.5
Sulphuric acid	13.0	13.7	13.6	9.9	10.6	10.6	12.2	13.3	11.2	9.0	10.0	7.8
Ammonium chlorid	11.1	11.1	12.8	7.9	7.8	9.9	11.0	10.8	12.2	7.5	7.9	9.0
Potassium hydroxid	14.0	12.1	12.3	11.0	9.2	9.2	11.8	11.3	10.8	8.6	8.3	7.9
Ammonium nitrate	10.7	12.6	11.6	7.3	9.3	8.4	10.6	10.7	13.0	7.3	7.2	9.7
Water		12.7			9.7			11.4			8.0	
Control		12.1			9.0			12.0			8.7	
Sodium chlorid	12.3	11.4	12.6	9.1	8.6	9.3	12.6	13.7	11.9	9.2	10.3	8.8
Calcium chlorid	12.5	12.1	12.8	9.4	9.2	10.4	12.9	12.0	12.4	9.7	9.0	10.1
Hydrochloric acid	12.1	11.6	11.6	9.2	8.9	8.8	12.2	12.9	14.3	9.0	9.5	10.9
Potassium sulphate	12.3	12.3	13.4	9.3	9.2	10.3	13.3	12.8	13.5	9.2	9.8	10.3
Magnesium nitrate	10.0	11.7	12.1	7.6	8.5	9.0	11.9	12.8	13.2	8.8	9.7	9.8
Magnesium chlorid	12.8	12.3	13.2	9.8	9.2	10.1	12.0	13.2	12.3	9.0	10.0	9.6
Nitric acid	10.3	10.9	10.8	7.0	7.4	7.5	10.3	11.2	11.6	6.8	7.9	8.7
Ammonia	.....	13.1	12.4	.....	9.7	8.9	.....	11.8	11.8	.....	7.8	8.8
Water		11.9			8.9			12.0			8.7	
Control		12.1			9.1			12.1			9.1	

RESULTS OF EXPERIMENTAL WORK

TOTAL YIELD AND PERCENTAGE OF GRAIN

The total yield represents the weights of the crops obtained immediately after they were harvested. The crops were harvested when fully ripe and after a continued dry period. The percentages of grain were determined in the samples, ranging from 1½ pounds to 2 pounds in weight, brought to Washington.

As seen from Table I, the plots which had received nitrogen in any form in the first stage gave distinctly higher yields, both as compared with the plots which had received no nitrogen and as compared with those which had received it in the other two stages. The application of the first stage was made later in the season than had been planned, and subsequent experiments have shown that the effectiveness of nitrogen with respect to increased yields diminishes as the season advances toward the completion of the vegetative stage.[5] On the other hand, as a result of the lack of uniformity in transition from stage to stage, at the time of the second-stage application part of the crops on each plot were still in the first stage. This probably accounts for the fact that the plots which had received their nitrogen in the second stage as a rule gave higher results than those which received it in the third stage.

The percentages of grain in the crops (Table I) were somewhat lower on the plots which had received nitrogen in the first stage than on the others. However, the lower range in the percentages of grain would not materially alter the grain relationship of the crops. The figures representing the total yields, therefore, are expressive of the relative yields of grain as well.

PERCENTAGE OF YELLOW-BERRY

The result of the lack of original uniformity in the plots, especially in those of series B, is most evident in the yellow-berry figures. Nevertheless, the fundamental principle that the application of nitrogen at the second stage is most effective in preventing yellow-berry was borne out very consistently. The percentage of yellow-berry is the lowest on the plots which received their nitrogen at the second stage. A marked decrease in yellow-berry is also shown by the crops which received their nitrogen in the first stage. This probably is due to the fact that the crops did not respond fully in yield because of a late application, and part of the nitrogen, applied at this stage and not utilized, remained in the soil during the second stage.

In some cases a somewhat decreased percentage of yellow-berry is shown by the crops which received their nitrogen in the third stage. This probably is due to the lack of uniformity in transition from one stage to another. At the time when most of the heads were in the milk stage, some of them had not yet reached this stage.

No conclusion can be drawn as to the effects of the direct and residual physiological acids and alkalies and their neutral salts, owing to the general lack of uniformity in the plots, which resulted in a too wide range of normal variation as indicated by control plots in both series, but especially in series B. Taking into consideration the fact that the

[5] DAVIDSON, Jehiel. THE EFFECT OF NITRATES APPLIED AT DIFFERENT STAGES OF GROWTH ON THE YIELD, COMPOSITION, AND QUALITY OF WHEAT. *In* Jour. Amer. Soc. Agron., v. 14, no. 4, p. 118–122. 1922

effect of nitrogen in preventing yellow-berry was shown clearly in spite of all the disturbing factors, one would be justified in assuming that the effect of these chemical groups was inconsiderable, if any. This would seem to settle the practical question as to the possibility of preventing the characteristic coloration of the grain associated with yellow-berry by means other than nitrogen. However, the theoretical question as to the nature of the coloration of flinty grain and of that affected with yellow-berry remains unsettled. It is still possible that the coloration is an indicator reaction in response to physiological alkalinity and acidity. All the ammonium salts probably underwent nitrification before they were assimilated by the plants. Their effect, therefore, would be the same as that of nitrates. It is possible also that the brown color of the flinty kernels is caused by the action of the alkaline amino groups which might increase with the general increase in the nitrogen content on the hypothetical natural indicator present in the hull.

WEIGHT PER BUSHEL AND WEIGHT PER 1,000 KERNELS

No correlation can be observed between the different treatments, which resulted in different nitrogen contents, and the weight per bushel and the weight per 1,000 kernels (Table III). Taking the plots in groups of three for each of the nitrogen carriers, it would seem that those which had received their nitrogen in the first stage gave a somewhat lower weight per 1,000 kernels than those which had received it in the second and the third stages. The first stage, however, is not the one which gave the highest protein contents. These results, as well as those obtained in Kentucky,[6] fail to disclose any direct relation between the protein content and the weight per bushel and, especially, the weight per 1,000 kernels. Cases are on record, however, in which such a correlation has been reported. Table IV, compiled from data reported in Bulletin 128 of the Bureau of Chemistry, United States Department of Agriculture,[7] will serve as an illustration. This table shows a reverse correlation between the nitrogen content and the weight per 1,000 kernels and weight per bushel for Kansas and South Dakota, but no correlation for California, as was the case in the experiments here reported.

What is the explanation of these seemingly contradictory results? Within the limits of the bureau's experiments, it has been established that the presence of nitrogen at the time of heading is conducive to a high nitrogen content. The conditions of these experiments would suggest the distinction between a physical and physiological abundance of available nitrogen in the soil. The term "physiological abundance" is used to signify a condition in which the crop, being depressed in its development, is kept from drawing upon the store of available nitrogen in the soil to the full normal extent. Any environmental conditions which depress the normal development of the crop cause a physiological abundance of available nitrogen. Under such circumstances, the factors which are responsible for the abundance of available nitrogen are responsible also for the subnormal development of the grain. Hence, the coincidence of a high protein content and a low weight per 1,000 kernels and a low weight per bushel. In these experiments, the abundance of

[6] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

[7] LECLERC, J. A. TRI-LOCAL EXPERIMENTS ON THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF WHEAT. U. S. Dept. Agr. Bur. Chem. Bul. 128, 38 p. 1910.

available nitrogen at the time of heading was distinctly physical, which may be the reason why no such correlation was found. It would seem, therefore, that there is no direct correlation between the protein content and the weight per 1,000 kernels and the weight per bushel of wheat.

PROTEIN CONTENT [8]

The results reported in Table V show that the application of nitrogen at the second stage gave the highest nitrogen contents. Although series A proved to be somewhat irregular, of the nine nitrogen carriers used six were consistent with the general tendency. Series B was wholly consistent. The irregularities, as well as the fact that increased nitrogen contents were obtained from the first and third application, can be explained in the same way as the irregularities with reference to yellowberry. The increased nitrogen content, which resulted from the first stage application, was due to the fact that not all the nitrogen was used up during the first stage. The increased nitrogen content resulting from the third stage application can be explained by the lack of uniformity in transition from one stage to another, already discussed. The same causes are responsible for the fact that the increases in nitrogen obtained from second stage applications are not as sharp as they were in the bureau's previous experiment.[9] While the first application showed an increase in nitrogen, due to the fact that not all the applied nitrogen had been utilized during the first stage, the nitrogen content from the plots which received their nitrogen at the second stage did not reach its proper height, for the reason that part of the nitrogen of this application was utilized in making vegetative growth as was shown by the increased yields from these plots.

The nitrogen content of the straw (Table VI) shows the same tendencies as that of the grain.

PHOSPHORIC-ACID CONTENT

Headden[10] found that the application of sodium nitrate to wheat plots caused a depression in the phosphoric-acid content of the grain. In the bureau's previous experiment,[11] where sodium nitrate had been applied on a number of plots at the three stages of growth, no correlation between the application of nitrogen and the phosphoric-acid content was disclosed. In the present experiment, however, a correlation is observed between the application of nitrogen and the phosphoric-acid content, both in the grain and in the straw. As seen from Table V, the phosphoric-acid content in the grain from the plots which received their nitrogen in the first and second stages is consistently lower than that in the grain from the plots which received it at the third stage and the controls which, in this case, may include all the plots which received chemicals other than nitrogen carriers. The only exceptions are the

[8] It is understood that the term "protein" represents values obtained by multiplying the nitrogen content, determined in the regular way, by certain factors. These factors vary with the substance analyzed. In the paper the factors used were 5.7 for the grain and 6.25 for the straw. It has been the general practice in agricultural literature to express the nitrogen values in terms of protein. This practice has been established because of the fact that the main bulk of the nitrogen in mature plant substance is protein in character and because of the accepted food value associated with proteins.

[9] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

[10] HEADDEN, W. P. A STUDY OF COLORADO WHEAT, PART III. Colo. Agr. Exp. Sta. Bul. 219, 131 p. 1916.

[11] DAVIDSON, J., and LECLERC, J. A. OP. CIT.

ammonium-nitrate plots in series A and the ammonia plots in both series. There would seem to be a tendency toward a greater depression in the plots of the first application. This tendency shows itself more clearly in series B.

On the other hand, results obtained on the Arlington Farm, in an experiment carried out on lines similar to these, again failed to disclose any correlation between the application of nitrogen and the phosphoric-acid content (Table VII).

It would seem, therefore, that there are conditions under which the application of nitrogen causes a depression in the phosphoric-acid content of the crop and that under other conditions the application of this element has no effect on the phosphoric acid content. What factors are involved in these seemingly contradictory tendencies?

Of the four cases cited here it happens that the two (Colorado and Nebraska), which show a correlation between the application of nitrogen and the phosphoric-acid content, deal with hard winter wheats and the other two (Kentucky and Arlington Farm), which show no such correlation, deal with soft winter wheats. This would suggest the possibility that these two groups react differently toward an abundance of available nitrogen. The underlying principle then would be, in the case of the hard winter wheats, some interchange between phosphorus and nitrogen, based on the similarity of their functions. The depressed phosphoric-acid content would be directly correlated with the high protein content. This assumption, however, does not seem to be borne out by the results in Table V. In those cases where the third application of nitrogen resulted in a high protein content, especially in series A, no depression in the phosphoric-acid content can be observed.

Another possible factor accounting for the different effects of applied nitrogen on the phosphoric-acid content is the supply of available phosphoric acid in the soil. It may be assumed, then, that when the supply of available phosphorus is limited the stimulation of growth, caused by the application of nitrogen, creates a physiological scarcity of this element. This assumption would seem to be borne out, in a general way, by the results here obtained. The application of nitrogen at the first two stages which resulted in increased yields also caused a depression in the phosphoric-acid content. There is also an apparent tendency toward a greater depression resulting from the first application, which produced the highest yields. This assumption, however, would not seem to be borne out by Headden's results. In his experiments the application of sodium nitrate did not cause an appreciable increase in yield, if any. Nevertheless, it produced consistent depressions in the phosphoric-acid content.

It is clear that under certain conditions the presence of nitrogen interferes with the assimilation of phosphoric acid by the plant. It is not unlikely that this interference is indirect. Under certain conditions the nitrogen may be instrumental, through its action on the soil flora, in transforming the available phosphoric acid into less available forms.

The phosphoric acid in the straw follows the same tendency as that in the grain (Table VI).

The ash and potash contents of the grain were also determined, but as the results failed to disclose any consistent tendencies they are not given

ASH AND SILICA CONTENT IN THE STRAW

As seen from Table VIII, the application of nitrogen at the first and second stages caused a distinct depression in the ash and silica content of the straw.

A depression in the silica content of the straw, caused by the application of sodium nitrate, was also observed by Headden.[12] In his case the depression was slight. In the bureau's experiments the difference between the first two stages and the third stage, for each nitrogen carrier, was always more than 1 per cent. The third stage plots, not being affected by the application of nitrogen with reference to the silica content of the straw, may be considered as controls. The sharper differences are due probably to the exceptionally high silica content of the straw in this experiment. Ammonia is the exception. It did not depress the silica content in either series. Attention is called to the fact that the same chemical also failed to produce a depression in the phosphoric-acid content of the grain. The relationship between the phosphoric-acid depression and the depression of silica in the straw is further accentuated by the fact that ammonium nitrate, which failed to depress the phosphoric acid in the grain from the second stage plot in series A, also failed to depress the silica content of the straw from the same plot. No explanation of the theoretical principles involved in depression of silica can be offered at present.

As to the bearing of this depressed silica content in the straw on lodging, frequently caused by the application of nitrogen, it is hard to draw conclusions from the present experiment. No lodging was observed on any of the experimental plots. It is possible that as the silica content of the straw in this experiment was exceptionally high, even the depressed silica content was sufficient to sustain the strength of the stalk. It is probable also that the depressed silica content is only one of the factors which cause lodging, or it may be no factor but only coincident with the real factors resulting from the application of nitrogen, which, under certain conditions, cause lodging of the crop.

SUMMARY

(1) The application of nitrogen in any of the inorganic forms used at the early stages of growth was instrumental in producing the highest yields of wheat.

(2) The application of nitrogen in any of the inorganic forms used at the time of heading was instrumental in producing the best quality of grain with reference to "yellow-berry" and the protein content. It also produced a high protein content in the straw.

(3) No relation between the nitrogen content and the weight per 1,000 kernels and weight per bushel of wheat was disclosed in this experiment. The apparent disagreement between these results and the results of other experiments, which establish a relation between these two factors, is explained by the distinction between a physical abundance and a physiological abundance of nitrogen created by the failure of the crop to develop normally.

(4) No differences could be observed in the effect of the different forms of inorganic nitrogen.

[12] Headden, W. P. op cit.

(5) No effect was produced by the chemicals, other than the nitrogen carriers, on the yield of the crop or on the quality of the grain.

(6) The application of nitrogen at the first two stages caused a depression of the phosphoric-acid content in the grain, as well as in the straw. In the light of these results, this depression could not be considered either as a yield relation nor as a reciprocal relation of the two elements in the plant tissues, based on similarity of function.

(7) The application of nitrogen, at the first two stages of growth, caused a marked depression of the ash and silica content in the straw.

POISONOUS PROPERTIES OF BIKUKULLA CUCULLARIA (DUTCHMAN'S-BREECHES) AND B. CANADENSIS (SQUIRREL-CORN)[1]

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INTRODUCTION

Since the time of the early settlements in the mountains of Virginia frequent fatal cases of poisoning have occurred among cattle grazing in the mountain pastures in early spring. It has long been believed that certain early spring plants popularly known as "staggerweeds" have been the cause of these fatalities, since these plants are among the first to appear in the pastures and are often eaten by cattle when other forage is not abundant. Suspicion has chiefly centered upon the plants commonly called larkspur (*Delphinium tricorne* Michx.), dutchman's-breeches (*Bikukulla cucullaria* (L.) Millsp.), squirrel-corn (*B. canadensis* (Goldie) Millsp.), and wild bleeding heart (*B. eximia* (Ker) Millsp.). In the literature relating to poisonous plants the toxic character of *D. tricorne* has long been recognized, but the American species of Bikukulla appear to have received comparatively little attention from chemists and practically none from toxicologists.

The probable poisonous character of species of Bikukulla was first brought to the attention of the Department of Agriculture in June, 1920, by Prof. H. S. Stahl, of the Virginia Polytechnic Institute. He submitted specimens of "little staggerweed," later identified as *Bikukulla cucullaria*, with the statement that this plant was believed to be responsible for the recent death of a number of cattle in the mountain pastures of Bland County, Va. An extract of the plant prepared in the laboratory was found to be highly toxic, and in order to secure material for further study a representative of the department visited the locality where the cases of poisoning had occurred. At the request of Dr. A. W. Drinkard, jr., Director of the Virginia Agricultural Experiment Station, arrangements were made to conduct some cooperative experiments with a view to determine by feeding tests the effect upon cattle of the plant material collected for chemical examination.

As a result of these investigations *Bikukulla cucullaria* has been shown to contain a poisonous alkaloid heretofore unrecognized, and the toxicity of this plant for cattle has been demonstrated. Poisonous alkaloids have also been found in *B. canadensis*, but this species is much less toxic than *B. cucullaria* and apparently is not likely to cause any damage to cattle.

[1] Accepted for publication Sept. 13, 1921. The plants referred to in the title are also known in botanical literature under the following genus names: Dicentra, Bicuculla, Dielytra, Diclytra, Corydalis, Fumaria, and others.

SYSTEMATIC POSITION OF "LITTLE STAGGERWEEDS"

BIKUKULLA

Perennial and smooth herbs with basal ternately compound, dissected delicate leaves; watery juice; horizontal rootstocks and scapose, racemose, nodding inflorescence. Flowers flattened, either deciduous or withering persistent. Pedicels 2-bracted. Sepals 2, scalelike. Petals 4, the two outer spurred at base, loosely united; the two inner pair narrower, and their callous-crested tips united over the stigmas. Stamens 6, in two groups opposite the outer petals, hypogynous; their filaments often united; middle anthers of each set 2-celled, the lateral ones 1-celled. Pod 10 to 20 seeds, seeds crested.

A genus of about 13 species, ranging across North America to Eastern Asia. Six species are known on the Pacific slope of America and 3 on the Atlantic watershed.

The bleeding heart (*Bikukulla spectabilis* (L.) Coville) of the gardens is an Asiatic species.

KEY TO ATLANTIC SPECIES

Racemes simple; rootstocks tuber-bearing.
- Rootstocks long, with scattered cornlike, yellow tubers; flowers cordate; spurs rounded; inner petals conspicuously crested.....1. *B. canadensis.*
- Rootstocks much shortened; tubers gathered in a scaly, granulated bulb, white, becoming pink and dark red; flowers sagittate; spurs spreading, elongated; inner petals minutely crested.....2. *B. cucullaria.*

Racemes compound; rootstocks scaly, not tuber-bearing.....3. *B. eximia.*

1. *Bikukulla canadensis* (Goldie) Millsp. Squirrel-corn. Turkey-corn. Turkey-pea. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Wild hyacinth.

Foliage bright green; scapes erect 6 to 12 inches high, overtopping the leaves; flowers greenish white, tinged with pink. On vegetable mold in woods. April and May. Nova Scotia, Ontario, Minnesota, Missouri, and south along the mountains to North Carolina and Tennessee. In Virginia it occurs abundantly on northern slopes in the Alleghany Mountains and often in the Blue Ridge.

It rises to 4,500 feet altitude on White Top Mountain, although it seems most at home in the neighborhood of 3,000 feet. Gorge of the Potomac above Washington. Rare.

2. *Bikukulla cucullaria* (L.) Millsp. Dutchman's-breeches. Little blue stagger. Trembling stagger. Staggerweed. Colicweed. Soldier's-cap. Whitehearts. Indian boy-and-girls. Butterfly banners, etc.

Foliage often glaucescent; scapes 6 to 12 inches high, usually overtopped by the leaves; flowers white with yellowish tips. April and May.

On vegetable mold in the woods. Growing in Virginia with *Bikukulla canadensis* and *Delphinium tricorne* but usually preferring better drained soil than the former. Nova Scotia, Ontario, Minnesota, Nebraska, Kansas, and Missouri, and south in the mountains to Alabama. Common on the northern slopes of the Alleghany Mountains and often in the Blue Ridge, being more abundant around 3,000 feet altitude and rising on White Top Mountain to about 4,500 feet. Gorge of the Potomac above Washington.

3. *Bikukulla eximia* (Ker) Millsp. Wild bleeding heart. Staggerweed. Turkey-corn.

Leaves dark green; scapes 1 to 2 feet high; longer than the leaves; flowers deep pink. Mountain rocks and river gorges, southern Appalachian Mountains from Wills Mountain, Allegany County, Md., south to North Carolina and Tennessee.

CHEMICAL EXAMINATION

As might be expected in a group so nearly related to the poppy family, species of Bikukulla have been found to contain alkaloids. Wenzell (*7*) [3] in a study of *Bikukulla canadensis* (*Corydalis formosa*) reports the presence of an alkaloid which he assumed, on very insufficient evidence, to be corydaline. Fischer and Soell (*3*) made an examination of *B. cucullaria* (*Dicentra cucullaria*) and were able to identify protopine and also to isolate minute quantities of two other alkaloids in crystalline form, which they characterized only by their melting points and certain other physical properties. Battandier (*2*) discovered protopine in *B. formosa* (DC.) Howell (*D. formosa*). Heyl (*5*) made a more thorough study of the same plant and observed that protopine was the chief alkaloid, besides isolating two others which he differentiated by their physical properties.

Gadamer (*4*) found protopine in *B. spectabilis* (*D. spectabilis*), with a yield of 1 per cent, and surmised the presence of other alkaloids. Asahina (*1*) obtained a crystalline alkaloid from *B. pusilla* (Sieb. & Zucc.) Coville (*D. pusilla*), which he named dicentrine, and also isolated protopine from the plant. He made a very complete chemical study of dicentrine and concluded that it was identical with one of the unnamed alkaloids found by Heyl in *B. formosa* having a melting point of 169° C. Dicentrine was studied from a pharmacological standpoint by Iwakawa (*6*), who found that it produced narcosis when administered to small animals in moderate doses, whereas larger quantities caused convulsions, weakening of the heart, and respiratory paralysis.

A search of the literature has failed to reveal any publication on the constituents of *Bikukulla eximia*.

Table I brings out the more essential facts in the preceding summary.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various investigators*

	BIKUKULLA CUCULLARIA, Fischer and Soell.		
Alkaloids	Protopine.		
Melting point	206° C.	231° C.	215° C.
Solubility	Soluble in alcohol. Soluble in ether.	Insoluble in alcohol. Soluble in $CHCl_3$.	Soluble in alcohol.
Form	Cryst. pr.	Needles, rosettes	Fine granular.
Color tests:			
Concentrated H_2SO_4.		Red to Brown.	
Erdmann's		Red to violet.	
Froede's		Red to violet.	
Concentrated HNO_3.		Red to yellow.	

[3] Reference is made by number (italic) to "Literature cited," p. 78.

TABLE I.—*Comparative chemical analyses of species of Bikukulla made by various investigators*—Continued.

BIKUKULLA FORMOSA, G. Heyl.

Alkaloids	Protopine		
Melting point	201° to 202° C.	168.5° C.	142.5° C.
Solubility		Soluble in H Br. salt. Insoluble in alcohol.	Soluble in H Br. salt. Soluble in alcohol.
Form	Needles	Yellow needles	White needles.
Color tests:			
Concentrated H_2SO_4.	Yellow	Colorless to red violet.	Colorless.
Erdmann's	Yellow to green	Blue	Weak green.
Froede's	Violet	Deep blue	Blue green.
Concentrated HNO.	Colorless	Colorless to brown	Brown.

BIKUKULLA PUSILLA, Y. Asahina.

Alkaloids	Protopine	Dicentrine.
Melting point	207° C.	168–9° C.
Solubility	Soluble in alcohol. Soluble in ether	Soluble in hot alcohol. Soluble in $CHCl_3$.
Form	Prisms	Prisms.
Color tests:		
Concentrated H_2SO_4	Yellow to blue	Colorless to violet.
Erdmann's	Yellow to violet	Blue.
Froede's	Violet to blue	Deep blue.
Concentrated HNO	Colorless to brown	Colorless to brown.

It will be seen from Table I that protopine seems to be the alkaloid most common to the members of the genus, and if Asahina's (*1*) supposition is correct dicentrine is common to *Bikukulla formosa* and *B. pusilla* and possibly may be contained in others of the group. It seems not improbable that the protopine of Heyl (*5*) and Gadamer(*4*), melting at 201° C., may be a different body from that of Fischer(*3*) and Asahina(*1*), melting at 207° C. Some of the work summarized above is very incomplete, with conclusions often based on color reactions, which are notoriously deceptive. The physiological action of these alkaloids had not been studied, with the exception of protopine, which has long been known as one of the opium group, and dicentrine in Iwakawa's(*6*) report. Neither of these two alkaloids would seem to possess sufficiently toxic qualities to account for the symptoms of poisoning in animals fed with *B. cucullaria* (Pl. 1, A) and *B. canadensis* (Pl. 1, B).

The material on which the following chemical work was done was gathered at Round Bottom, Va., in April, 1921, just previous to the flowering stage. The tops and roots of *Bikukulla cucullaria*, and only the roots of *B. canadensis* were used in the experiments. The tops of the *B. cucullaria* were dried and in good condition, but the roots of both plants were contaminated with soil which was removed by sieving in a stream of running water. They were then dried, first in the air and then in an oven at 100° C., and ground fine in a mill.

The object of the study here reported was to determine what constituents of the plants were responsible for their toxic effect, and as it was known that both contained alkaloids it was obviously necessary to isolate and test the toxicity of these compounds. As a preliminary experiment a few grams of the ground roots of the two plants were allowed to macerate in Prolius' solution for 48 hours. The solution was then decanted and evaporated spontaneously until nearly dry, and the residue was extracted with dilute hydrochloric acid. Both these solutions gave a heavy precipitate when tests were made with Mayer's reagent, showing the presence of alkaloids. The acid solutions were then made strongly ammoniacal, which threw down some of the dissolved material as a white flocculent precipitate, and were shaken out exhaustively with ether. After the ether evaporated, the residue was again taken up with very dilute hydrochloric acid. One-half cc. of each of these two solutions, which represented the raw alkaloids of *Bikukulla cucullaria* and *B. canadensis*, was injected subcutaneously into two white mice. The animal receiving the dose of *B. canadensis* gave some slight evidence of narcosis but suffered no other ill effects. The mouse injected with the *B. cucullaria* extract also behaved in a sleepy manner for some time and died unobserved inside of two hours. Another mouse was then given a similar injection of the same *B. cucullaria* extract, and his reactions were carefully followed. The animal showed no striking symptoms in the course of an hour, then suddenly grew restless for a few minutes, was seized with acute convulsions, and died almost immediately. These results seemed to indicate that the roots of *B. cucullaria* were poisonous, whereas those of *B. canadensis* were harmless, but upon further investigation this conclusion was modified.

An approximate assay of the total alkaloids in the material which was available, namely, the tops and roots of *Bikukulla cucullaria* and the roots of *B. canadensis* was undertaken as follows: Of each of these three samples 5 gm., dried and finely ground, were macerated for 48 hours in 50 cc. of Prolius' solution, the solution was decanted off and the residue washed with a little alcohol and added to the solution, which was then extracted with 50 cc. of 5 per cent H_2SO_4 in three portions. The acid extract was made alkaline with ammonia and shaken out exhaustively with ether. The ether solution was distilled to small bulk, transferred to a tared beaker, and allowed to evaporate spontaneously, whereupon it was completely dried in a desiccator and weighed. Table II shows the results of the three determinations.

TABLE II.—*Assay of total alkaloids found in Bikukulla cucullaria and B. canadensis*

Plant.	Weight of plant.	Alkaloid.		Character of residue.
			Per cent.	
B. cucullaria:	*Gm.*	*Gm.*		
Tops	5	0.062	1.24	Amorphous.
Roots	5	.080	1.60	Amorphous, with some needles.
B. canadensis:				
Roots	5	.157	3.14	Amorphous, with rosettes.

With known weights of the raw alkaloids from definite quantities of the three samples, the way was now open for comparative tests of their toxicity. To this end all three total alkaloidal residues were dissolved in exactly 5 cc. of very dilute acetic acid, and 0.5 cc. of each was injected subcutaneously into three mice. The result was the same in each instance the animal died almost instantly in violent convulsions. The dosage was as follows:

B. cucullaria:
Tops.....0.5 gm. plant=0.0062 gm. alkaloid.
Roots.....0.5 gm. plant= .0080 gm. alkaloid.
B. canadensis:
Roots.....0.5 gm. plant= .0157 gm. alkaloid.

By testing these solutions at gradually increasing dilutions it was possible to determine within narrow limits the minimal fatal dose of the three with respect to mice of about 20-gm. weight, as shown in Table III.

TABLE III.—*Minimal fatal dose of Bikukulla cucullaria and B. canadensis for mice of 20-gm. weight*

Plant.	Weight of plant.	Alkaloid.	Total dose per kilogram of body weight.
B. cucullaria:	*Gm.*	*Gm.*	*Gm.*
Tops	0.040	0.0005	0.02
Roots	.031	.0005	.02
B. canadensis:			
Roots	.250	.0100	.40

From these figures it can be estimated that the combined alkaloids of *Bikukulla cucullaria* roots and those of the tops are of approproximately the same degree of toxicity.

This finding runs counter to observations made in the field tests on calves, as reported elsewhere, in which 60 pounds of the tops were fed without effect. A possible explanation of this result is that the bulk and succulence of the fresh tops so dilutes the poison that its action is minimized and it is absorbed so gradually that the animal suffers no ill effects. Nevertheless the fact remains that the tops contain the poison and must be held partially accountable for the poisoning of grazing stock which eat the whole plant. Applying the foregoing figures to a comparison of the relative toxicity of the roots of *Bikukulla cucullaria* and *B. canadensis*, it will be seen that the alkaloids of *B. cucullaria* are about 20 times as poisonous as those of *B. canadensis*, but as there is a considerably larger percentage of alkaloids in *B. canadensis* the ratio of toxicity between the two plants would be reduced to approproximately 6 to 1.

As a preliminary experiment to acquire some information about the character of the alkaloids in the two plants under investigation, 100 gm. of each were extracted in a Sohxlet apparatus with the selective solvents, ether, chloroform, alcohol, and acidified water. In the case of *Bikukulla cucullaria* the acidified water gave only the faintest of tests with Mayer's reagent, showing that the other three solvents had made a complete extraction. All three solutions contained appreciable quantities of alkaloids. From the chloroform and alcohol fractions only amorphous

products were obtained, but from the ether extract a crystalline alkaloid was isolated. After the ether was distilled off the residue was triturated with dilute hydrochloric acid until all the alkaloid was removed and the acid solution was made ammoniacal and shaken out with ether. The etherial solution was concentrated to small bulk, the same volume of alcohol was added, and on slow evaporation there were deposited warty masses of colorless crystals. These were removed and pressed between filters and recrystallized from a mixture of alcohol and chloroform. After one recrystallization they melted at 168° C., which was not changed by a further purification of the same kind. It was at once assumed that the dicentrine of Asahina (*1*) had been isolated, but after testing the compound on mice it was evident that it was another substance, and one of much higher degree of toxicity. By methods already described, it was found that 0.04 mgm. of the alkaloid was fatal to a mouse. The animal exhibited a period of intense excitement followed by convulsions and death, whereas Iwakawa in trying out dicentrine on mice found the fatal dose to lie between 5 and 10 mgm., and his animals experienced a prolonged period of narcosis before death. These facts would seem sufficient to differentiate the two compounds.

Another alkaloid is known with a melting point of 169° C., namely, Y-homochelidonine, found chiefly in *Sanguinaria canadensis* L. It has always been prepared in conjunction with its physical isomeride B-homochelidonine, melting point 159°, but no trace of the latter was found in our product. Moreover, the physiological action of Y-homochelidonine is like morphine, chiefly narcotic. It is therefore reasonable to assume that the alkaloid isolated from *Bikukulla cucullaria* is new, and we propose to name it provisionally cucullarine. Unfortunately, the small quantity at our disposal has made it impossible to make a complete study of it, but this we intend to do later when larger quantities of material are available. We have been able, however, to note the following properties: It crystallizes in characteristic warty masses of prisms; it is colorless when first prepared but changes to pink in the light; it is soluble in ether and chloroform, but not so soluble in alcohol; it is precipitated by the usual alkaloid reagents, Mayer's, iodin in potassium iodid, picric acid (crystalline), and platinum chlorid; with concentrated sulphuric acid a small crystal dissolves with difficulty, producing a violet coloration turning brown.

Another portion of *Bikukulla cucullaria* was exhaustively extracted with 95 per cent alcohol containing a little acetic acid, the alcohol was removed by vacuum distillation, and the residue was taken up in dilute acetic acid and allowed to stand for some time. The small quantity of resin which separated was filtered off, and the solution was made alkaline with ammonia and shaken out first with ether then with chloroform. Very little alkaloid was recovered from the latter extract, the great bulk being removed by the ether. The etherial solution was set aside for several days with the expectation that protopine would crystallize out. This did not occur, however. The ether was then distilled off, and the raw alkaloid fractioned in various ways with the production only of amorphous products. Our failure to isolate protopine, reported by Fischer and Soell in *B. cucullaria*, may be due to the comparatively small quantities of material on which the work was done or to the stage of maturity of the plant. Fischer and Soell do not say when their *B. cucullaria* was gathered and are rather obscure in their description of the technic they employed.

As with *Bikukulla cucullaria* it has not been possible as yet to give the alkaloids of *B. canadensis* a complete survey. It seems highly probable that the latter plant contains cucullarine, as the physiological effects produced on mice by the raw alkaloids of both plants are so similar. If present in *B. canadensis* it undoubtedly must be there in very minute quantity, as the toxicity of *B. cucullaria* is so much greater. This may account for the failure so far to isolate it. However, a crystalline alkaloid from the roots of *B. canadensis* has been prepared. The material was percolated with 95 per cent alcohol, plus acetic acid, and the solution was treated in the usual way. By extracting this preparation with ether which contained a small quantity of alcohol a bright yellow compound was obtained, crystallizing in bundles of silky needles. It has a melting point of 210° C. and gave reactions with the customary alkaloid reagents and a crystalline precipitate with picric acid. It proved to be nontoxic when injected into a mouse. A dose of 0.6 mgm. caused the animal no apparent inconvenience.

It is proposed to make a further study of the alkaloids of *Bikukulla cucullaria*, *B. canadensis* and *B. eximia* in more detail with larger quantities of material.

FEEDING EXPERIMENTS

Feeding experiments with both *Bikukulla cucullaria* and *B. canadensis* were made by the representative of the Virginia Agricultural Experiment Station on a farm near Merriam, Bland County. The material used was collected on this farm and fed in a fresh condition. The animals used were healthy yearling steers, weighing approximately 275 pounds each.

At first these plants only were fed, but the animals ate of them so sparingly that it was found necessary to mix them with grass before they were taken freely. Account was kept of the weight of the grass and of the plants fed to each animal and of the portion which remained uneaten, from which the approximate weight of the suspected plants eaten was calculated. A detailed account of the experiments follows.

STEER 2.—The whole plant of *Bikukulla cucullaria* was given this animal. Feeding was begun at 2 p. m., April 19, 1921, but on that day only ½ pound of the plant was eaten; April 20, 3½ pounds were consumed between 7.30 a. m. and 5 p. m., without noticeable effects. Feeding was resumed at 5.15 p. m.; and at 5.30, when the animal had eaten about another pound of the plants, symptoms of poisoning were first exhibited. He suddenly began to tremble, ran backward then forward several times with the head held very high. He was frothing at the mouth and several times ejected partly digested stomach contents a distance of several feet. The trembling which occurred all over the body became more violent, convulsions ensued, and at 5.40, 10 minutes after the first seizure, he fell and was unable to rise. He lay upon the left side, with the head thrown back as in opisthotonos, and with the legs extended and rigid. He moaned as though in great pain, the eyes were glassy, breathing was very difficult, and the bowels were lax. About 5.50 relaxation began; the animal struggled but was unable to rise until 6.10, when he got up; and although very weak and nervous he was able to walk about. From this time his condition improved rapidly, and, when no other symptoms of poisoning appeared, he was returned to the pasture at noon, April 21.

STEER 5.—The bulbs alone of *Bikukulla cucullaria* were fed to this animal. Feeding was begun at 2 p. m., April 19, but practically none of

the material was eaten until the following day when approximately 5 pounds were consumed between 7.30 a. m. and 5.30 p. m. Then trembling, convulsions and all other symptoms manifested by steer 2 were exhibited. As in the case of steer 2 this aninmal was eating heartily of the bulbs at the time of seizure.

There was no appreciable difference in the time both animals were down or in their condition during recovery. These two cases agree so closely in every respect that they are regarded as practically identical.

STEER 4.—Between April 19 and April 25 this animal received 60 pounds of *Bikukulla cucullaria* tops which included the flowers. There were no ripe seeds in the material. The quantity consumed daily ranged from 4 to 11½ pounds. No symptoms of poisoning were observed during the seven days the animal was under observation.

STEER 3.—This animal consumed approximately 55 pounds of the entire plant of *Bikukulla canadensis* between 5.30 p. m., April 19 and 12.30 p. m., April 27. The quantity consumed daily ranged from 3½ to 9½ pounds. Aside from a slight restlessness and uneasiness on the second day of feeding, when 7½pounds of the plant were eaten, no injurious effect was observed.

STEER 6.—This animal was brought in for feeding on the afternoon of April 24, and was given the entire plant of *Bikukulla canadensis*. Feeding was continued until noon, April 27, up to which time he had consumed 16 pounds of this material. Six and one-half pounds of the plant were eaten on April 24, and on April 25 the animal was restless and uneasy practically all day, but no other symptoms were manifested.

These experiments confirm the popular opinion that *Bikukulla cucullaria* is poisonous to cattle, but more definite feeding experiments must be performed with *Bikukulla canadensis* before it can be positively stated that this species constitutes a menace to live stock.

CONDITIONS UNDER WHICH POISONING MAY OCCUR

Most of the cases of poisoning occur early in the spring. The danger is usually past by the middle of May, although in some of the higher altitudes poisoning may occur as late as June. Poisoning is said to occur most frequently following heavy rains, when, the soil being soft, the bulbs of the plant are mostly likely to be pulled up and eaten. The experiments show that both species of *Bikukulla* used are unpalatable to cattle and therefore unlikely to be eaten in harmful quantities when suitable forage is available.

SUMMARY

(1) *Bikukulla culcullaria* and *B. canadensis*, in Virginia popularly called "little staggerweeds," have long been considered poisonous to cattle.

(2) Chemical examination has shown that both these plants contain toxic alkaloids and that the tops as well as the bulbs of *B. cucullaria* are poisonous.

(3) *B. cucullaria* contains at least one alkaloid of a highly poisonous nature. This alkaloid, heretofore apparently unknown, has been named cucullarine, and its properties are described.

(4) Cucullarine probably occurs in *B. canadensis* also, since its physiological effect on mice closely resembles that of *B. cucullaria*.

(5) Feeding experiments show that *B. cucullaria* is toxic for cattle.

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PLATE I

A.—*Bikukulla cucullaria*, Dutchman's-breeches.
B.—*Bikukulla canadensis*, squirrel-corn.

B

A

FORCING THE GERMINATION OF FRESHLY HARVESTED WHEAT AND OTHER CEREALS[1]

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In the regions where the winter wheat harvest precedes sowing by only a few weeks, as in Wisconsin, farmers depend largely upon the current crop for fall sowing. The inability to secure a prompt and satisfactory germination test of the freshly harvested grain, when tested at the ordinary temperature, 20° C. or a little higher, has heretofore created an administrative difficulty, which became acute toward the end of the sowing season when a prompt report by the analyst was imperative. The work reported in this paper was done in an attempt to overcome this difficulty.[2] In all, about four dozen samples of wheat and one dozen each of barley and oats of the current crop, part of them gathered from the standing grain, were included in the investigation. More than half of these samples were gathered by hand in the State of Wisconsin. Others were sent there or to Washington from Colorado and from the Dominion of Canada.

Germination tests were made in 100-mm. Petri dishes with moist absorbent cotton as a seed bed, using only unbroken grains which showed no visible evidence of decay or injury. The majority of the samples were presoaked about half an hour in Gooch crucibles in running tap water, those badly infected with microorganisms being first sterilized from two to five minutes in a 1 per cent solution of silver nitrate. Only 50 grains were used in the majority of the tests, but 100 or 200 in some tests. Preliminary germination tests of all samples and a few special tests in which temperature effects were not important were made at room temperature (about 20° to 26° C.). Samples which germinated very well in the preliminary tests were not included in any of the tests with different methods of forcing germination.

On account of the limitations under which the work was done, it was necessary to count grains as germinating while in the very early stages of germination and to discontinue the majority of the tests at the earliest possible date. This practice was justified by observation upon further development at a favorable temperature for growth in a large number of selected cases.

Moisture determinations were made concurrently with the germination tests by drying small samples of the grain for two or three days in an oven in which the temperature was maintained at about 105° C. and the air was kept in circulation by a small electrically driven fan.

[1] Accepted for publication July 2, 1921.

[2] The greater part of the work was done in Madison, Wis., during August, 1919, with the cooperation of Prof. A. L. Stone, State seed inspector of Wisconsin, on whose suggestion the investigation was undertaken, Dr. W. W. Robbins, who at that time was with the Colorado Agricultural Experiment Station, and the Canadian analysts. Prof. Stone assisted in the arrangements for collecting samples in Wisconsin. Dr. Robbins and the Canadian analysts sent samples from their localities for test. The necessary equipment was furnished by the Branch Cereal Laboratory of the United States Department of Agriculture at Madison and the Department of Plant Pathology at the University of Wisconsin. After my return to Washington, Miss Bertha C. Hite, of the Seed Testing Laboratories, continued the work, concerning herself especially with the effect of increased oxygen pressure and the germination of old cereals at different temperatures.

A great deal of work has been done by others on the after-ripening of seeds in general and more with the cereals than with any other class of seeds on account of their economic importance. Much of this work with the cereals by English and German investigators has been related to the adaptation of fresh grain for use in the brewing industry, although relations to agricultural practice and breeding have not been entirely neglected. Many and various hypotheses have been put forward to explain the process of after-ripening, but some of these lack convincing experimental evidence and none is of universal application.

RESULTS OF THE INVESTIGATION

EFFECT OF ARTIFICIAL DRY HEATING

Duchartre (*15*),[3] working with spring rye, wheat, and barley, was one of the first to call attention to the beneficial effect of artificial drying on the germination of seeds. He found that cereal seeds were capable of germination when they were still far from mature and their endosperms were just leaving the milk stage; that very young seeds germinated more slowly, but with as high a total percentage as the fully ripened grain; that artificial drying so affected these very young seeds that they germinated about as rapidly as the mature grain; and that such artificially dried young grain was satisfactory for seeding purposes.

Since Duchartre's publication, the New York (Geneva) Agriculture Experiment Station (*40*), Hotter (*24*), Hoffman (*22*, *23*), Hiltner (*21*), Atterberg (*6*), Kiessling (*28*), and Stapledon and Adams (*39*) are among those who have published results showing favorable effects upon germination from drying not after-ripened cereals and maize. Zade (*47*) found only slight advantage from artificial drying of wild oats when fresh, but when the dormant seeds which had been in moist sand for months were removed from the sand, dried, and remoistened a very large percentage germinated promptly.

Recently Kondo (*30*) has shown beneficial effects from drying not after-ripened rice. He considers this to be due to an increase in the oxygen supply made possible by a change in the oats. This explanation seems to be applicable to Zade's favorable results with wild oats, since Atwood (*7*) has shown that oxygen is the limiting factor in the germination of these seeds.

In nearly all the published work on artificial drying to hasten the after-ripening and germination of cereals the drying has been done by means of heating, and the results are therefore complicated by a possible effect of the heating per se. In fact Kiessling, in the article already cited, attempted to show that the beneficial effects he secured were really temperature effects and not effects of fluctuations in moisture content at all. However, Hiltner (*21*) and others have shown an accelerating effect upon after-ripening as a result of drying in vacuo or over sulphuric acid. The writer, in conjunction with Dr. William Crocker in work as yet unpublished, accelerated after-ripening of Johnson grass seed by drying over lime or over sulphuric acid, but the effect developed much more slowly and less completely here than when the seeds were heated in a drying oven. Apparently in this case both heating and drying are beneficial and the result seems to be related to the subsequent water intake of the embryo.

[3] Reference is made by number (italic) to "Literature cited," p. 97–100.

The effect of drying and heating treatment depends to an extent not always recognized upon the condition of the grain at the time of treating it, the temperature used, and the length of time the treatment is applied. Kiessling (*28*) found a beneficial effect upon barley in the early stages of after-ripening and a harmful effect in the later stages or after after-ripening was complete. Stapledon and Adams (*39*), with very mild dry heat treatments, secured beneficial results with good strong grain and harmful results with weak or diseased grain. Atterberg (*6*) reported a decrease in germinating capacity as a result of drying for a short interval, followed by an increase with longer drying. Wollny (*45*), working with fully afterripened cereals, found that artificial drying decreased germination but increased the productivity of the resulting plants. According to Worobiew (*46*) heating wheat before germination promoted a xerophytic type of structure and increased productivity under drought conditions but had no effect with ample moisture supply. Apparently Johannsen's failure (*25*) to induce the germination of fresh two-row barley and Stapledon and Adams' (*39*) only slightly favorable results with a number of cereals are to be explained as a result of drying for an insufficient length of time.

The temperatures used for drying have varied from about 30° C. to as high as 80° C., and the results with the higher temperatures have frequently been harmful on account of the relatively high moisture content of the cereals at the time the heating was begun. Müller (*36*), Waggoner (*42*), Harrington and Crocker (*20*), Nagai (*37*), Atanasoff and Johnson (*5*), and others have shown that cereal and other seeds are quite resistant to the effect of temperatures even as high as 100° C. if their moisture content is low when the heating commences. Zalenski (*48*) found 50° C. for a short time fatal to fresh green grain of winter rye and harmful to the grain in later stages of maturation.

Several have found a temperature around 40° C. to be best for increasing the germination capacity of not after-ripened cereals. Stapledon and Adams (*39*) used this temperature for three days with limited effect. Hotter (*24*) gave 10 days, Hiltner (*21*) 8 to 10 days, and Atterberg (*6*) 8 days as the length of time drying at 40° C. was necessary to bring not after-ripened cereals to their full germinating capacity. Atterberg obtained the same results from 8 days' drying at 30° C. if the seeds were dried in a current of air, a fact which seems to indicate that oxygen supply or rate of drying is important. Moritz and Morris (*34*) gave 43° C. as the maximum temperature to be used in kiln-drying barley for malting, and Hiltner stated that temperatures above 40° C. may be injurious to the fresh, relatively moist not after-ripened grain. Since barley is more resistant than the other cereals to the harmful effects of high temperatures 40° C. would seem to be as high a temperature as could safely be adopted for general use.

In the author's investigations heating for one day at 40° C. was without effect upon the germinating capacity of very moist, fresh wheat. Table 3 shows the effects of other dry-heat treatments upon germination at room temperature.

Drying wheat eight days at 40° C. had a much more pronounced effect upon its subsequent germination than drying at the same temperature for five days. This drying treatment was more effective in hastening germination with barley than with wheat or oats, but it raised the germination capacity to nearly 100 per cent in all cases. When the temperature

was raised to 62° after eight days at 40°, and the drying was continued for one day, the rapidity and completeness of germination of oats and wheat were reduced to about the figures for the undried controls. Barley, after the same treatment, germinated even better than when dried only at 40°. This agrees with the statements of others that barley is more resistant to the injurious effects of high temperature than are the other cereals and in addition seems to indicate that up to the point at which injury begins, increasing the drying temperature would increase the effectiveness of the treatment.

TABLE I.—*Effect of artificial heating and drying*

Kind of grain and number of samples.	Drying treatment.	Average moisture content in percentage of wet weight.	Percentage of germination.			
			Average of all samples.		Samples showing greatest effect of the treatment.	
			2 days.	5 days.	2 days.	5 days.
Wheat, 3 samples	Control, not dried	12.3	[a] 61	69	[a] 52	6
	Dried 5 days at 40° C	8.1	71	78	68	7
Wheat, 4 samples	Controls, not dried	12.4	38	78	32	6
	Dried 8 days at 40° C	6.9	59	95	54	9
Oats, 6 samples	Controls, not dried	11.6	20	85	8	6
	Dried 8 days at 40° C	5.5	27	98	20	9
Barley, 3 samples	Controls, not dried	12.3	9	29	16	2
	Dried 8 days at 40° C	6.1	71	99	86	[a] 10
Wheat, 2 samples	Controls, not dried	12.3	47	83	32	7
	Dried 8 days at 40° C	7.0	68	93	54	8
	Same+1 day at 62° C	4.8	38	73	24	5
Oats, 5 samples	Controls, not dried	11.6	20	83	8	6
	Dried 8 days at 40° C	5.5	27	98	20	9
	Same+1 day at 62° C	4.7	8	92	0	7
Barley, 1 sample	Controls, not dried	12.3	8	36	.....	.....
	Dried 8 days at 40° C	5.7	58	100	.....	.....
	Same+1 day at 62° C	4.7	80	100	.....	.....

[a] In 3 days.

Tests of several of the samples which showed poorest germination in five days were continued two days longer. Germination continued at a decreasing rate during this time, but was far from complete at the end of the time in case of some of the untreated lots, especially of barley, and some of the lots of wheat and oats which had been heated at 62° C.

EFFECT OF PRESOAKING

Bleisch (*9*), with barley which germinated well without treatment, and Andrews and Beals (*3*), with fully after-ripened maize, have shown acceleration of germination as a result of presoaking. Hiltner (*21*) and Atterberg (*6*) greatly increased both germinating energy and germinating capacity of a number of kinds of freshly harvested cereals by presoaking combined with a pricking of the endosperms. Kiessling (*28*) decided that presoaking not after-ripened cereals at various temperatures had a uniformly harmful effect as compared with drying at the same temperatures and that this harmful effect was not entirely overcome by the

"heat stimulus," which he makes the basis upon which rests the beneficial results of both drying and presoaking. With water as the heating medium and the grain therefore in a sensitive condition the heat stimulus is felt in a few hours, whereas in dry condition days are required. Too long heating (overstimulation according to Kiessling (*28*), is of course harmful. Moufang and Vetter (*35*) therefore recommended soaking barley one hour at 45° C., followed by soaking in the cold in preparing it for malting. Too long-continued soaking at any temperature also is recognized as harmful by all who have worked upon the subject, and Wollny (*45*), Kidd and West (*27*), and others have emphasized the fact that harmful effects result from presoaking in an excess of standing water. This danger is reduced by using running water. According to Kidd and West (*27*), presoaking the red dwarf bean in excess of water increased the rate and vigor of germination but decreased the total percentage germinating and the number and dry weight of the seedlings which survived after three weeks in good soil. The injury thus measured and the amount of exosmosis from the seeds while soaking was greater when they were soaked at 5° to 10° C. or at 30° C. than when soaked at 20° C.

Braun (*10*) has recently described in connection with the treatment of cereals for seed-borne diseases a successful method of presoaking which is said to stimulate germination and early growth at the same time that it increases the efficiency of the sterilization treatments. Braun used a minimum quantity of water and limited the soaking time.

Wollny (*45*), Kraus (*31*), and Hiltner (*21*) reached conflicting conclusions, which Eberhart (*16*) attempted to harmonize, as to the effects of presoaking cereals and other seeds upon the resulting crop yield. All these except Hiltner (*21*) agreed that presoaking under certain conditions has a very marked effect in increasing crop yield. The seeds were sometimes sowed moist and sometimes after a period of drying, which did not seem to destroy the beneficial effects. Characteristic differences were observed at different stages of development between the plants from the controls and those from the treated seeds. Wollny (*45*) attributed the effects to coat changes which allow a more rapid water intake and initiate a more vigorous growth, though the percentage germinating is usually lessened, as Kraus (*31*) also decided. Eberhart (*16*) agreed that the permeability of the seed coats to water is increased by the presoaking as also by favoring ether treatments and that this increase is important; but he concluded that protoplasmic alterations also must be involved, since the effect is not temporary but persists throughout the growing period.

As already stated, the majority of the germination tests in this investigation were made after soaking the grain about one-half hour in running water. The grains to be tested for germination were placed in the bottoms of Gooch crucibles, which were then stacked in columns of not more than 12 in ring-stand supports. Tap water at approximately 18° C. from a spigot which gave a spattering stream, so that the water must have been well charged with atmospheric air as it bathed the grain, was then allowed to run through the entire columns. The object of this presoaking was to wash the seeds as free as possible of microorganisms and also to remove or precipitate (by means of chlorids in the water) the silver nitrate remaining on the grain in those tests in which the grain had been sterilized. There is no reason to believe that there were any harmful effects from this treatment.

One sample each of fresh wheat, oats, and barley was soaked in ta water at room temperature 1 hour and 5 hours in lots of 50 seeds each which were then put to germinate at 16° and at 25° C. During the soaking the seeds were covered about 1 inch deep with water. The germination time was reckoned from the time when the soaking was begun Table II shows the results of the germination tests.

One hour's presoaking greatly increased and 5 hours' presoaking slightly increased the germination within the first 31 hours, but presoaking even for 1 hour greatly reduced the percentage which germinated in 4 days in case of oats and barley but did not affect it in the case of wheat. The harmful effect of the presoaking was much greater when the subsequent germination test was made at 25° C. than when the germination temperature was 16°.

TABLE II.—*Effect of presoaking 1 and 5 hours in excess of standing water upon subsequent germination*

Kind of cereal.	Germination temperature.	Hours soaked.	Percentage germinating.	
			31 hours.[a]	4 days.
	°C.			
Wheat	16	0	0	10
		1	76	10
		5	12	9
	25	0	10	6
		1	16	7
		5	16	6
Barley	16	0	0	9
		1	46	6
		5	4	6
	25	0	4	7
		1	34	4
		5	8	1
Oats	16	0	0	9
		1	28	5
		5	26	5
	25	0	26	9
		1	26	3
		5	8	3

[a] Reckoned from the time the soaking was begun.

In order to get an idea of the progress of imbibition as affected by presoaking, 50 seeds of known weight of one poorly germinating sample each of wheat, oats, and barley were soaked one hour in small vials after which the water was poured off. The grain was emptied upon a blotting paper, the surface water was rapidly removed by rubbing with another small piece of blotting paper, and the percentage of increase in weight was determined in comparison with control lots on moist blotting paper in Petri dishes. The presoaked grain was then placed on moistened blotting paper in Petri dishes, from which it was removed from time to time and its increase in weight determined in comparison with the control lots. During the first eight or nine hours the grain was at room temperature, and thereafter in an incubator at 5° C. to prevent germination. Table III shows the percentage of increase in wieght of these lots of grain up to the end of the third day.

The rate of imbibition during the first hour was very much greater for the presoaked lots than for the controls. The presoaked oats continued absorbing water faster than the controls for several hours on wet blotters, while the presoaked wheat was absorbing water faster at the end of 72 hours than the control. With all three kinds of grain the difference in moisture content at the end of the presoaking period was practically maintained up to the end of the three days. There was scarcely any tendency for the moisture content of the soaked lots and the controls to become equalized within that time. This seems to indicate that the thorough wetting of the surface, displacing the surface film of air, in the case of the presoaked grain had established favorable conditions for the capillary movement of water over the surface of the grain toward the proximal region at which Schroeder (*38*) and Collins (*12*) have shown that nearly all water intake occurs and tended to increase permanently the imbibitional power of the presoaked grain. Wheat showed less difference between presoaked lots and controls than did barley or oats.

The high rate of water absorption during and immediately following the period of presoaking probably accounts for the early beginning of germination of presoaked grain, and undoubtedly the thorough filling of coat structures with unaerated water retards gaseous exchange, explaining the reduction in germination capacity resulting from presoaking in excess of water. It is, however, impossible to consider coat limitations to the total water-absorbing power as the cause of poor germination of the freshly harvested grain, since the quantity absorbed by even the control lots by the end of the third day must be far in excess of the minimum amount required for germination.

TABLE III.—*Percentage of increase in weight on wet blotters of grain presoaked 1 hour in comparison with controls not soaked* [a]

Number of hours.[b]	Percentage of increase in weight.					
	Wheat.		Oats.		Barley.	
	Presoaked.	Control.	Presoaked.	Control.	Presoaked.	Control.
1	15.7	8.9	38.7	13.1	25.4	8.4
2	21.3	13.4	46.7	16.1	32.2	15.7
4	29.5	23.3	59.8	26.1	42.3	28.4
8½	40.0	34.1	75.7	37.3	56.6	36.5
24	[c] 46.6	40.1	80.4	47.2	63.5	46.2
48	53.6	46.7	87.9	57.4	69.2	55.4
72	58.3	[c] 50.4	86.6	[c] 60.8	72.0	[c] 60.8
Moisture content after 72 hours (percentage of dry weight)	81.4	71.4	114.0	84.3	100.0	83.9

[a] Grain held for first 8 or 9 hours at room temperature, then at 5° C.
[b] Times given are only approximate for oats and barley, but the time was always the same for a presoaked lot and its control.
[c] One or more beginning to germinate.

EFFECT OF STERILIZATION WITH SILVER NITRATE SOLUTION

For use in the comparative germination tests at different temperatures to be discussed in a later section, grain of the samples which were badly infected with microorganisms—about one-half of the total number of

samples—was sterilized for five minutes in a 1 per cent solution of silv nitrate [4] with subsequent, thorough washing. Concurrent comparati germination tests of a number of samples with and without previo sterilization of the grain showed that the sterilization had no effect upo germination except to increase that of grain which molded badly witho such sterilization.

Additional trials showed that wheat with not more than 10 to 12 p cent moisture could, with impunity, be immersed two minutes in a 1 p cent silver-nitrate solution even after cutting off the distal end, provide that the washing was very thorough. The cut surfaces of the endosper subsequently darkened but germination did not suffer. A small pe centage germinated even after the embryo had been scratched with needle followed by two minutes in 1 per cent silver nitrate and thoroug washing. Of course, a majority of the grains thus treated could n escape serious injury. This would be the case also with grains cracke or mutilated by thrashing or handling.

INFLUENCE OF THE SEED BED UPON GERMINATION

The result of the imbibition experiments and of the germination tes after presoaking suggested an attempt to secure by the use of a suitab germinating bed as much as possible of the accelerating effects of th presoak without its undesirable effects upon germinating capacity.

Trials were therefore made in 100-mm. Petri dishes with one rathe poorly germinating sample each of wheat, barley, and oats, using see beds of different character and moisture content.

For seed bed the following were used: (1) Four circular disks of heav blue blotting paper; (2) raw cotton of low absorbing capacity; (3 Johnson & Johnson absorbent cotton. When blotting paper disks wer used the grain was tested both on top of the four disks and between th second and third disks.

Four blotting paper disks weighed from 7½ to 8 gm. It required fro 12 to 13½ cc. of water to saturate them and then 1 cc. additional mad sufficient excess to drip when the uncovered Petri dishes were a littl more than half inverted. This additional amount of water was a absorbed by the blotters in the next two or three hours. For the germi nation tests between blotters just the amount of water required to satu rate the blotters was used in each case. For the germination tests o top of the blotters 1 cc. was added.

The Johnson & Johnson absorbent cotton in each dish weighed onl 1.8 gr. (less than one-fourth as much as the blotters) and was saturated when firmly pressed down, with 11 cc. of water. The addition of 6 cc of water was then not sufficient to allow any to drip when the uncovere dish was a little more than half inverted. Thus the water-holdin capacity of the absorbent cotton was greater than that of four times a great weight of the blotters; and of this large amount of water held b the cotton without flooding the seeds five or six times as much wa readily available for rapid absorption by the seeds.

In the germination tests the amount of water required to saturate th cotton (11 cc.) was used in comparison with other tests using 2 cc. les and 6 cc. more water.

[4] A solution of silver nitrate is a very desirable medium for surface sterilization on account of the eas with which it can be removed or precipitated as the chlorid, the tenacity with which the silver chlorid held on the coat structures, and the consequent relatively permanent sterility conferred.

The same weight of raw cotton was used as of the Johnson & Johnson cotton (1.8 gr. in each dish). Into this was carefully worked as much water as it could be made to hold, and the excess was then squeezed out and poured off. The amount retained could not be accurately determined but was about 10 cc. in each dish.

Table IV gives the result of the germination tests at 20° C. Fifty seeds were used in each test.

With barley and oats concurrent tests were made at 10°, 13° 17°, and 25° C. The best results, especially as to rapidity of germination, were obtained between saturated blotters and on top of the supersaturated absorbent cotton. Raw cotton and the only partially saturated absorbent cotton gave very poor results. In additional tests upon absorbent cotton using somewhat more than 17 cc. of water, the harmfull effects of flooding the grain with an excess of water became evident. The quantity used should not be enough so that any can be easily poured off from the Petri dishes, and the grains should be allowed to rest lightly upon the cotton instead of being pressed into it so that the water surrounds them.

The wheat was less sensitive to moisture conditions than the oats and barley, and the results were conflicting. Some other samples, tested at other times, seemed to be more sensitive than the one used in this series, and the optimal moisture conditions were the same as for oats and barley.

In germination tests at 10°, 13°, and 17° C., the percentage of germination varied less, according to seed bed and amount of moisture used, than at 20°, being between 90 per cent and 100 per cent in nearly all cases. The relation between moisture supply and rapidity of germination, however, was the same as at 20°, germination being most rapid between blotters and on top of supersaturated absorbent cotton and least rapid on raw cotton and partially saturated absorbent cotton. At 25° germination was very poor and the effect of moisture supply was greater than at 20°.

At all temperatures, the best root development was obtained between blotters and the most rapid epicotyl development on top of supersaturated absorbent cotton.

TABLE IV.—*Germination at 20° C. on different seed beds and with varying amounts of moisture*

Seed bed.	Cc. water.	Percentage germinated.								
		Wheat.			Barley.			Oats.		
		3 days.	5 days.	8 days.	3 days.	5 days.	8 days.	3 days.	5 days.	8 days.
On top of blotters	13–14.5	36	76	80	20	40	40	24	92	92
Between blotters	12–13.5	56	76	82	28	68	72	58	88	92
Raw cotton	[a] 10	22	84	92	4	32	32	16	76	76
J. & J. cotton	17	58	88	92	60	64	64	48	92	92
J. & J. cotton	11	46	88	88	16	52	52	24	72	84
J. & J. cotton	9	64	90	94	4	32	32	16	80	88

[a] About.

EFFECTS OF MECHANICAL TREATMENTS

1. WOUNDING

Hiltner (*21*), Atterberg (*6*), and Kiessling (*28*) improved the germination of various freshly harvested cereals by pricking or cutting the endosperms. Similar results were obtained by Zade (*47*) and by Atwood (*7*) with not after-ripened wild oats, by Harrington (*19*) with not after-ripened Johnson grass seed, and by Andrews and Beals (*3*) with fully after-ripened maize. Hiltner (*21*) and Atterberg (*6*) combined the wounding treatment with presoaking, to which the results were in part attributed. Various interpretations of the favorable effects of the wounding have been put forward.

In wild oats, Atwood (*7*) showed that the effect of the wounding is undoubtedly due to increased oxygen intake, oxygen deficiency being responsible for inability to germinate. Atwood's results with cultivated oats also indicate that increased oxygen supply may be involved in the effect with oats. Hiltner (*21*) believed that the wounding increases germination by allowing imbibition of sufficient moisture, and others have spoken of enzymic activity being initiated by the admission of oxygen and the oxidative transformation of proteins.

Kiessling (*28*) discredited all explanations of after-ripening which are based on coat exclusions. Since immediately closing the wounds with paraffine did not prevent the favorable effect of the wounding he believed that the effect of wounding is reached by exerting a "stimulus" upon the living protoplasm as in his interpretation of the effects of heat and cold on a stimulus basis. Behrens (*8*) came to the same conclusion, and Zade's dissertation also includes some data which might be thought to favor the idea that the dormant embryo of wild oats is in a state of exceedingly delicate equilibrium and responds readily to mechanical shocks. But in the case of Zade's work, it is probable that the rubbing of the embryo, which he interprets as causing mechanical stimulation of the embryo protoplasm, in reality increased the permeability of the coats to oxygen, thus supplying the increased amount of oxygen which Atwood later showed to be necessary. Furthermore, Kiessling's stimulus hypothesis is difficult to accept on account of the mechanical obstructions in the path of conduction to the embryo of any wound stimulus exerted upon the endosperm. The only path through the medium of living cells seems to be by way of the aleurone layer, which has no organic union with the embryo.

In the investigations herein reported two methods of wounding were adopted, both of which had been found to be very effective in causing the germination of dormant Johnson grass seed (*19*), namely, (1) cutting off the distal end of the grain just back of the embryo and germinating the embryo end alone, and (2) scratching the embryo itself along the entire length of one side of the scutellum by means of a bent dissecting needle. The axial organs were avoided, but the scutellum was rather deeply wounded. Only a few lots were treated in either of these ways.

When the distal ends were cut off and discarded the embryo ends of all lots of barley and oats and all but one lot of wheat so treated showed nearly complete germination at room temperature in from three to six days, but a marked tendency to decay after the treatment reduced the percentage of healthy seedlings from some unsterilized lots below that for the controls. One lot of wheat showed 100 per cent germination in

three days after treatment, against 56 per cent in three days and 64 per cent in five days in the control. The only lot which failed to germinate well after treatment was a lot of scarcely ripe wheat freshly harvested from the standing plants and carrying nearly 30 per cent (wet-weight basis) of moisture. This lot germinated 16 per cent in six days after treatment and failed to germinate at all in the control. Probably if comparable lots of oats and barley had been treated in the same way the germination would have been no better.

Simply removing the hulls from poorly germinating lots of oats and barley slightly increased their germination.

Scratching the embryo had much more effect than cutting off the distal end, inducing practically complete germination in two or three days of all lots thus treated except the one lot of wheat which was least affected by cutting of the distal end. This germinated 86 per cent in six days, while the control failed to germinate at all. The tendency to decay was much less than when the endosperms were removed, apparently on account of germicidal properties of the wounded embryos.

2. REMOVAL OF THE COAT STRUCTURES WITH CONCENTRATED SULPHURIC ACID

This treatment also has been found very successful with dormant Johnson grass seed (*19*). In this investigation it was used only with wheat. It is classed as a mechanical treatment because its effect apparently depends entirely upon the removal or weakening of the coat structures over the embryo. The grain was immersed in the concentrated acid for from 30 seconds to 5 minutes, washed in sodium-bicarbonate solution, rubbed free from as much as possible of the disintegrated tegumentary structures, and finally washed for about half an hour in running water. The first part of the coat to be visibly affected was that over the radicle, which became slightly charred in 30 seconds. After 3 minutes' treatment nearly the entire coverings rubbed off readily except over the edges of the scutellum and over the distal end of the caryopsis. Even after 5 minutes these parts were not wholly bared. The most rapid and complete germination was secured after 3 to 5 minutes' treatment, but the acceleration was quite noticeable when the treatments lasted only 30 seconds, therefore only slightly weakening the coat but probably effecting its permeability to a marked degree. This was the most effective of all the mechanical treatments, causing complete and prompt germination of the most resistant sample; but it is almost as tedious as the others and entails great danger of subsequent decay.

In all probability, the effect of all mechanical treatments here reported depends fundamentally upon the same cause, which may be either (1) relieving the axial parts of the embryo of some inhibiting substance by diffusion outward, or (2) an increase in respiration or an alteration in its nature by allowing more ready exchange of oxygen and respiratory products, or (3) a more obscure "stimulus" to the living protoplasm. Possibly, also, a similar stimulation of the epithelial layer of the scutellum plays at least a secondary part when the endosperm end of the caryopsis is removed or the scutellum is wounded by scratching. In this connection, it should be said that simply rubbing off the thin membrane which remained over the embryo of barley after the imbibed naked caryopses were removed from the scales also induced quick and complete germination of the most troublesome sample. The embryos were injured, however, sufficiently to cause some of the elongating coleoptiles to curve

abnormally upward on account of more rapid growth on the under than on the upper side. The possibility of a wound stimulus is therefore not eliminated.

GERMINATION AT DIFFERENT TEMPERATURES

Atterberg (*6*) was perhaps the first to call attention to the favorable effect of cool temperatures upon the germination of not after-ripened cereals. He considered after-ripening as simply the continuation of the normal process of ripening, and recommended that, with cereals too unripe to germinate well at from 13° to 15° C. the germination test be preceded by drying for eight days at 40° C. He also found that the use of alternating temperatures sometimes increased germination of such grain. Kiessling (*28*) got better germination at 12° to 16° C, than at 18° to 23° C, as late as November and better at 10° to 14° C. than at 18° to 22° C. until late stages of after-ripening, when the comparison was reversed. Recently, Stapledon and Adams (*39*) stated that from 12° to 15° C, gave best results with nearly all samples of fresh cereals. Of course at the low temperatures the rate of development is very slow and germination therefore has to be counted very "closely" or else the germination test has to be continued over a longer period.

An alternative is to transfer the grain to a higher temperature for an additional day or two as soon as practically all have begun to germinate.

Table V summarizes the results of a large number of germination tests at different temperatures.

The very favorable effects of temperatures well below 20° C. is strikingly evident. With oats and barley the highest average total percentages of germination in two weeks were secured at 5° and 9°. There were very considerable differences in response to temperature between individual samples of each kind of cereal.

TABLE V.—*Germination at different temperatures*

AVERAGE PERCENTAGE BEGINNING TO GERMINATE AT DIFFERENT TEMPERATURES

Kind of cereal.	Number of samples.	Number of days.	5° C.	9° C.	12° C.	16° C.	19° C.	22° C.
Wheat	16	2	5	54	89.5	89	88	52
		5	94	99	99	99	95	85.5
Oats	7	2	1	2	26	41	37	28
		5	32	69	79	71	65	55
		11–14	87	88	85	77	72	68
Barley	3	2	0	1	3	17	13	5
		5	28	63	67	63	45	24
		11–14	95	94	86	80	.....	.....

PERCENTAGE OF GERMINATION OF SAMPLES SHOWING MAXIMUM TEMPERATURE EFFECT

Wheat	1	2	0	0	50	40	22	8
		5	88	96	98	84	40	16
Oats	2	5	44	48	84	94	60	50
		5	82	94	92	68	52	48
Barley	1	5	20	80	86	80	56	20

A number of the poorer germinating samples of wheat were kept in the germinators for two weeks. With these, as with oats and barley, nearly all the grains previously ungerminated began to develop at the lower temperatures, but the germination of several samples remained far from complete at 19° C. and especially at 22°.

When barley and oats were kept in the cool germinators for the longer period, the endosperms usually became fluid and milky, while the later appearing germinations were often abnormal and weak. It is questionable, therefore, whether these should be counted as germinated. Atterberg's (*6*) combination of artificial drying with subsequent germination at cool temperature should be used with such samples. No such doubt is attached to the result with wheat, or to the early appearing germinations of barley and oats.

A temperature somewhat lower than 20° C. is of advantage also in making germination tests of older samples of cereals. Probably about 16° is the optimum. The percentage of germination of old samples frequently is slightly greater, and never is significantly less than at 20°, the development of the seedlings is sufficiently rapid, and the tendency to mold or decay is markedly less than at 20°. The results of this investigation show this to be true with oats and wheat, and incidental references in the literature on germination indicate that it is probably true also of barley and rye.

Several samples of spring wheat were very poorly developed on account of the rust epidemic, the selected grains for some samples being hardly more than half normal weight. Nevertheless, these samples responded as well to the mechanical treatments described in the previous section and to low temperatures as did the well-matured samples.

The use of relatively low germinating temperatures for winter cereals has the added advantage of simulating much more nearly the conditions under which the grain would germinate in the field. Planted in the fall, it has cool nights at least, and soon the average soil temperature, even in the day, is well below 20° C. Furthermore, Aderhold (*1*), Appel and Gassner (*4*), Gutzeit (*18*), Gassner (*17*), and Walster (*43*) have shown that the exposure of different kinds of seed or very young seedlings to low temperatures—always well below 20° C and frequently around 0°—has a very marked effect upon the subsequent development of the plant. In the case of cereals (especially winter cereals) this effect is decidedly beneficial, since it promotes an early, uniform, and abundant fruitage. The formative effect of temperature upon the plastic organs of plants has recently been further illuminated by Child and Bellamy (*11*). All of these investigations taken together open up a very interesting field for further study in connection with the after-ripening and germination of cereals.

EFFECT OF INCREASED OXYGEN PRESSURE UPON GERMINATION

As indicated in earlier pages Atwood (*7*) has found increased oxygen pressures efficient in forcing the germination of dormant wild oats, Kiessling (*28*) obtained similar results with tame oats and also found oxygen treatments helpful in increasing the germination of barley in the early stages of after-ripening but harmful in the later stages of after-ripening. Kondo (*30*) concluded that after-ripening in rice consisted of a process of oxygen storage and enzym formation and that the beneficial effect of drying was related to increase oxygen entrance. Hoffman

(*23*) elaborated a similar hypothesis, so far as oxygen is concerned, to explain the after-ripening of cereals.

On the other hand, Kiessling, in the article just referred to, showed that barley after-ripened more slowly in atmospheres in which two-thirds of the normal air was replaced by oxygen than in unaltered atmospheric air, and also that the beneficial effect of heating, either with or without simultaneous drying, was not dependent upon the partial pressure of oxygen during the period of heating. Takahashi (*41*), Nagai (*37*), and Akemine (*2*) have all shown that fully after-ripened rice germinates and develops considerably in entire or almost entire absence of oxygen, and Lukas (*33*) found that fully after-ripened oats and maize, as well as some other seeds, germinated more rapidly in reduced air pressure (therefore reduced partial pressure of oxygen) than in normal air. The behavior of rice is undoubtedly related to its ecology, this grain being adapted for germination under water, as are the seeds of many water plants (*13*, *14*).

From Kiessling's (*28*) and Lukas's (*33*) results, it would seem either that the oxygen requirements of the embryos decreased during after-ripening, or else that increasing permeability of the coat structures during the after-ripening rendered oxygen in a given partial pressure more available for the use of the embryo. Atwood's (*7*) results show that the latter is probably the case with wild oats, while the work of the Japanese workers favors the former explanation with rice.

In this investigation, increased partial pressures of oxygen were used in testing the germination capacity of a number of not after-ripened samples of wheat. The apparatus used consisted of a small battery jar inverted within a larger battery jar so that two or three litres of gas was imprisoned over a water seal. The grain was tested on moist blotting paper in open Petri dishes floated on large corks within the small inverted battery jars, using 100 grains for each test. Increasing the percentage of oxygen in the atmosphere to 36 per cent had apparently the maximum effect on germination. With this percentage 85 per cent of one sample of wheat germinated in five days against 44 per cent with the normal 20 per cent of oxygen, while the amount of growth was nearly twice as great. With higher partial pressures of oxygen, the percentage of germination was about the same as with 36 per cent, but the rate of growth was less.

WATER CONTENT IN RELATION TO GERMINATION

Some of the earlier workers on after-ripening of cereals and the effect of artificial drying attempted to establish a relation between the moisture content of the seeds or alternations therein immediately preceding the germination test on the one hand and germination on the other hand. As shown in a previous section, artificial drying has usually had a beneficial effect. However, Atwood (*7*), Kiessling (*28*), Kondo (*30*) and others have shown in articles already cited that after-ripening takes place also when loss of water is prevented or even when more water is absorbed by the grain during the period of after-ripening. Besides the frequently favorable effect of presoaking as well as of drying is opposed to the view that the germinating capacity of not after-ripened grain is quantitatively related to its water content at the time the germination test is made. The relations involved go deeper, are less simple, and involve many other factors than moisture.

The author has found no quantitative relation between moisture content and germinating capacity. For instance, some samples of wheat collected from the shocks with 12 to 13 per cent moisture content germinated well at room temperature soon after collection, before their moisture content had fallen appreciably, while other samples collected and tested at the same time under the same conditions and with the same moisture content at the beginning of the test germinated poorly. Under proper treatment or after a period of after-ripening the latter germinated as well as the former.

In Table VI the germination data for a number of samples tested at the same time at room temperature are arranged according to the moisture content of the samples on moist-weight basis at the time the test was begun. Although there is a general falling off of germination with increasing water content, the water content groups overlap in regard to germination. All of the 11 samples falling in the last column (greater than 14 per cent moisture) were collected from the standing grain and tested within six days after collection. The relations between moisture content and germination are further illustrated by the data for individual samples in Table VII.

RATE OF AFTER-RIPENING: POSSIBLE VARIETAL DIFFERENCES

Attberg (*6*) stated that cereals may pass the entire winter in ordinary dry storage without becoming fully ripe (using "ripe" in the sense that he considers after-ripening as but a continuation of normal ripening processes), and that 1 or 2 months' drying at room temperature may be necessary. Kinzel (*29*) stated that oats reached their full germinating capacity in 2 months, but their full germinating energy only after 8 or 10 months.

TABLE VI.—*Germination of freshly harvested cereals of different moisture content in 5 days at room temperature*

	At moisture content of—				
	10 to 11 per cent.	11 to 12 per cent.	12 to 13 per cent.	13 to 14 per cent.	Greater than 14.
Wheat:					
Number of samples	8	6	9	6	6
Percentage of germination—					
Average	95	93	73	59	26
Maximum	100	100	100	98	52
Minimum	86	84	26	28	0
Oats:					
Number of samples	0	1	3	1	2
Percentage of germination—					
Average	.....	70	75	48	64
Maximum	.....	.....	90	.....	90
Minimum	.....	.....	52	.....	38
Barley:					
Number of samples	0	0	1	2	3
Percentage of germination—					
Average	.....	.....	100	100	11
Maximum	.....	.....	.....	.....	16
Minimum	.....	.....	.....	.....	8

TABLE VII.—*Variety, length of time harvested, moisture content, and germination of wheat samples*

Sample No.	Variety.	Number of days since cutting.	Percentage of water.	Percentage of germination.
W_2	Winter	34	12.5	94 in 4 days.
W_3	do	34	13.4	86 in 4 days.
W_7[a]	Winter (Wisconsin No. 2)	34	13.3	42 in 5 days.
W_8[a]	do	34	12.8	56 in 5 days.
W_{11}	do	34	13.3	98 in 2 days.
W_4[a]	do	30	.....	28 in 5 days.
W_5[a]	do	30	13.4	40 in 5 days.
W_9	Spring (Kruger's)Wisconsin Wonder)	25	12.9	100 in 2 days.
5902	Winter (Turkey Red)	24	10.8	94 in 2 days.
5900	do	23	10.3	92 in 4 days.
W_6	Spring (Blue Ribbon)	17	13.2	52 in 5 days.
W_{10}	Spring (Marquis)	17	12.9	26 in 5 days.
W_{12}[d]	do	2	28.9	10 in 6 days.
W_{13}[b]	do	4	20.4	16 in 5 days.
W_{14}	Volunteer in oat field	4	15.0	52 in 5 days.
W_{15}	do	5	17.1	24 in 5 days.
W_{16}[c]	Spring	6	23.8	50 in 5 days.
W_{17}[c]	do	5	14.7	14 in 5 days.

[a] W_7 was from the outside and W_8 from the inside of the same bunch; W_4 from the top and W_5 from the bottom of the same bunch.

[b] W_{13} was collected from same field as W_{12} but was greener.

[c] W_{17} was from the same field as W_{16} but was standing and was somewhat better grains than W_{16}. W_{16} had been cut the preceding day.

[d] W_{12} to W_{17} were collected from standing grain on a rainy day and stored in the heads in heavy envelopes until the day of the test for germination.

Table VII presents such data as are available regarding rate of after-ripening arranged according to the length of time which had elapsed between cutting the grain or collecting the samples and the first germination test. The tests were made simultaneously at room temperature. On account of the lack of time, none of these samples were systematically retested at intervals to keep track of the rate of after-ripening.

There is no quantitative relation between the lapse of time since cutting and germinating capacity. The data suggest such varietal differences as have been described by Kiessling (*28*), who found striking differences, constant from year to year, in the initial dormancy and rate of after-ripening of different varieties and pure strains of wheat, oats, and barley and related these differences to differences in winter hardiness in case of winter wheat. Some varieties of barley after-ripened fully in two weeks, while others required more than two months. So far as the data in Table VII go, they indicated that Turkey Red winter wheat and especially Kruger's Wisconsin Wonder spring wheat are quickly after-ripening varieties; Marquis spring wheat and Wisconsin No. 2 winter wheat are slowly after-ripening varieties. According to Kiessling's (*28*) observations, the quickly after-ripening varieties, germinating sooner and more uniformly and becoming better established before the advent of cold weather, are also those recognized as the more hardy varieties. On this basis, Turkey Red ought to be more resistant to severe winters than Wisconsin No. 2, which is a strain isolated from Turkey Red stock. This condition is the reverse of what actually obtains in Wisconsin but is a matter which would undoubtedly vary in different localities and with grain from different sources. This suggested relation ought to be followed up in further investigations.

DISCUSSION

The work reported in this paper does not warrant an attempt to discuss in any detail the processes involved in after-ripening of the cereals or the numerous hypotheses previously offered to explain either these processes or the unanalyzed fact of after-ripening. Previous work by others has been cited when it was sufficiently related to the work herein reported. The enzymic theory of after-ripening has been almost entirely neglected in the references to the literature, not because this theory does not deserve mention but because the investigation did not touch on that phase of the general problem. The principal aim has been to present the results which are of value in the practise of seed testing and to fortify these results by citations of the results of others rather than to illumine or to explain the physiology of after-ripening or of germination.

All of the treatments which were of value in increasing the germination of the incompletely after-ripened cereals may logically be considered in connection with their oxygen and water requirements if we consider the effect of dry heating to be to increase the permeability of coat structures to oxygen. But there is no proof that heating has this effect. It may act in a more direct way on the embryo. In like manner in the other beneficial treatments effects upon the embryo protoplasm are not always out of the question. The bulk of the evidence does, however, seem to favor an explanation in which oxygen and water relations are involved and coat effects are important. Oxygen relations appear to be especially important. Since the cereals investigated seem under any conditions to absorb much more water during the germination test than the minimum required for their germination and since increased oxygen forced germination it seems probable that moisture and imbibition effects were related to the effects upon oxygen relations and respiration. Since the grain can be forced to germination and vigorous growth at any time, it does not seem that a storage of oxygen is a necessary part of after-ripening as postulated by Hoffman (*23*). The more probable explanation is that the treatments which force germination merely bring about the necessary increase in oxygen supply by removing coat structures or increasing their permeability to oxygen and that this change in permeability of the coat occurs also in normal after-ripening. Kiessling's (*28*) results with heating, with and without loss of moisture and in atmospheres of various compositions, upon which his stimulus hypothesis of the effect of heating is based are at least as readily explained on the basis of coat permeabilities. This is the situation which Atwood (7) found with wild oats, but it does not hold good for rice with which there must be either a storage of oxygen or a decrease in oxygen requirements during after-ripening.

Windisch (*44*), neglecting coat factors, lays emphasis on cell-wall permeability factors within the grain, which influence osmotic movements of nutrients. Windisch's hypothesis is not in consonance with the ready germination of the fresh grain when coat structures are removed or weakened.

The interrelations between moisture content, temperature, coat characteristics and changes therein, oxygen intake, rate of imbibition, rate and character of respiratory exchanges, response of the protoplasm to wounding and to the other treatments, changes in food reserves, the formation and activation of enzyms during the period of after-ripening and under both normal and forced germination ought to be thoroughly investigated, as well as the effects of the different treatments so far as

possible upon seedling vigor, upon the development of the resulting plant, and upon crop yield. The work of Kidd and West (26) in bringing together under the head of "Physiological predetermination" the result of previous work bearing on the latter subject, as well as the work referred to on previous pages, emphasizes the probability that the periods of maturation and germination of the seed, and perhaps also the intervening period of after-ripening, may be about the most impressionable stages in the life history of the plant for the reception of influences which are capable of modifying fundamentally the whole future course of its development. In future investigations along this line possible varietal differences should always be kept in mind.

SUMMARY

(1) Newly harvested cereals frequently do not germinate well at 20° C. or above under the ordinary conditions for germination tests. This fact has caused difficulty in administering State seed laws in the winter-wheat areas where harvesting precedes sowing by only a few weeks and the current crop is used for seeding.

(2) The embryos of the cereals investigated are never essentially dormant, dormancy being imposed by coat structures.

(3) Artificial dry heating, opening the coat structures over the embryo with incidental wounding of the scutella, and cutting off the distal ends, were effective in varying degree in inducing the germination of not after-ripened or partially after-ripened wheat, oats, and barley at room temperature.

(4) Removal or weakening of the coat structures over the embryos of wheat by the use of sulphuric acid was exceedingly effective in inducing complete germination in a minimum length of time.

(5) Artificial drying, to be completely effective, must be continued for a week or more and thus unduly delays the germination tests.

(6) Removal of the scales from oats and barley increased their germination somewhat, and removing the loose pericarp and tegumentary structures over dormant barley embryos caused complete germination at room temperature.

(7) Increased oxygen pressure in the atmosphere greatly increased the germination at room temperature of partially after-ripened wheat.

(8) The mechanical treatments, wounding and corrosion with sulphuric acid are very tedious processes and entail great danger of subsequent decay.

(9) Presoaking for even one hour in excess of water accelerated the germination of partially after-ripened wheat, oats, and barley but decreased the total germination. The soaking increased the rate of imbibition, and apparently the injurious effect upon total germination was the result of limiting gaseous exchanges by saturation of the surface layers of the grain with unaerated water. Presoaking half an hour in running aerated water did not seem to have any ill effect.

(10) For the germination of not after-ripened cereals, the seed bed should be such as to supply abundant water available for rapid absorption by the grain, but to do this without flooding the grain. Somewhat supersaturated absorbent cotton in Petri dishes satisfies these requirements if the grain is placed lightly on top of the cotton. Barely saturated blotters give equally good results if the grain is placed between the blotters, but not if the grain is placed on top of the blotters.

(11) A temperature considerably lower than 20° C. is much more satisfactory than 20° or higher for the germination of freshly harvested wheat, oats, and barley. Wheat responds to the low temperatures more uniformly than oats or barley. Nearly all fresh samples of wheat can be satisfactorily tested for germination at from 12° to 16° without previous treatment and without undue loss of time. With barley and oats the time required is longer, and it may sometimes be desirable to precede the germination test by dry heating.

(12) There is no quantitative relation between water content and germinability. After-ripening progresses at the same time as normal loss of water during curing of the grain, but not primarily as a result thereof. It occurs also at a modified rate when water loss is prevented or slight water absorption is caused.

(13) Some samples after-ripen much more quickly than others. The rates of after-ripening may be varietal characteristics and may be related to winter hardiness under some climatic conditions.

(14) Oxygen relations appear to be very important in the germination of not after-ripened cereals. The beneficial effects of mechanical treatments and of artificial drying and heating are probably related to increased oxygen supply to the embryo. It seems likely also that the permeability of coat structures to oxygen increases during after-ripening and that this increased permeability is related to the improved germination.

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A NEW AND EFFICIENT RESPIROMETER FOR SEEDS AND OTHER SMALL OBJECTS: DIRECTIONS FOR ITS USE[1]

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PREVIOUS RESPIRATION APPARATUSES

In the study of the dormancy, after-ripening, and germination of seeds, quantitative studies of their respiration are of major importance. For such studies on seeds and other small objects, a great many devices have been used, but none of them is perfectly adapted for the kind of studies the authors desired to make. Hence, the attempt to devise a new apparatus which would meet our requirements. Some of the apparatuses previously used, with their limitations, are indicated below. They are grouped according to the principle involved in the method.

(1) DEVICES FOR ABSORBING WITH CAUSTIC THE CARBON DIOXID (CO_2) RESPIRED INTO A STREAM OF CO_2-FREE AIR, OR OTHER GAS OR MIXTURE OF GASES, WHICH IS CONSTANTLY BEING DRAWN THROUGH THE APPARATUS CONTAINING THE RESPIRING MATERIAL, WITH SUBSEQUENT DETERMINATION OF THE AMOUNT OF THIS ABSORBED CO_2 BY TITRATION OR BY FILTERING AND WEIGHING THE CARBONATE

Among the earliest, most widely known, and most generally used of these devices are the long inclined tubes first used by Pettenkofer (*21*),[2] and later somewhat modified by Pfeffer (*22*). These tubes, with or without modification, and combined with appropriate special devices for incubation of the experimental material, and for controlling the pressure when necessary, have been used by a large number of investigators in important studies on carbon assimilation and normal and intramolecular respiration. Winkler's modification in Hempel (*11*) of the Pettenkofer tube consists in bending it into the shape of a spiral so that it is easier to use.

Other devices which have been much used for the absorption of CO_2 in a stream of air which had been drawn through the receptacle containing the respiring material consist of vertical towers of caustic solution. The tower invented by Reiset (*23*) has three multiperforate disks of platinum inserted to interrupt the stream of air and break it up into small droplets, thus insuring complete removal of the CO_2. Reiset tubes have been used by Brown and Escombe (*3*) in their important work on the energetics of green leaves, and by many others.

Recently, Gurjar (*7*) has adopted Truog's absorption tower, first used in soil analyses, for use in respiration studies. This tower consists simply of a vertical glass tube, ground into the neck of the flask which carries the caustic solution and filled to any desired height with glass

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[2] Reference is made by number (italic) to "Literature cited," p. 113–115.

beads which break up the current of air as it rises and take the place of the platinum disks of the Reiset tubes.

Sachs (in Grafe, *6*) drew the respired air through a series of simple absorption bottles, Kostytschew (*13*) used Giessler bulbs, and many other devices have been used by others.

With sufficiently large quantities of actively respiring material, apparatuses of this type are excellent for determining one side of the respiratory reaction. They have the advantage of avoiding the disturbing effects of changes in the partial pressures of oxygen and CO_2, but they neglect entirely the question of the amount of oxygen absorbed by the respiring material, which is quite as important as, and not always in proportion to, the amount of CO_2 given off.

Furthermore, they are not adapted for use with limited quantities of very small or relatively inactive seeds, as the amount of CO_2 given off in reasonable time may in this case fall within the limits of error of the method. Unless special precautions are observed, the experimental errors are likely to be rather large.

(2) ABSORPTION BY CAUSTIC SOLUTION OF THE CO_2 RESPIRED IN A CLOSED SPACE WITH OR WITHOUT SIMULTANEOUS MANOMETRIC MEASUREMENT OF THE OXYGEN ABSORBED

The apparatus used by Wolkoff and Mayer (*32*) was simply an inverted U-tube, one arm of which dipped into a dish of mercury, while the other arm was much enlarged and received the plant material and a dish of caustic solution. This end of the tube was, of course, closed during the experimental period, and the whole apparatus was immersed in a water bath.

Godlewski (*5*) cultured his material in a closed flask which contained a dish of caustic solution and was connected with a one-arm open manometer dipping into mercury. Stich (*24*) modified this apparatus for effective temperature control by immersion in a water bath.

When the manometer is used, this type of apparatus gives both oxygen consumption and CO_2 production, but since the CO_2 is absorbed as rapidly as it is given off and does not therefore compensate for the oxygen absorbed by the respiring material, the method often involves relatively large changes in pressure during an experimental period; these pressure changes might in some cases affect the results. Besides, like the preceding type of apparatus, this type requires special care to avoid errors due to the absorption of CO_2 from sources other than the respiration of the material being studied and is not adapted for use with limited quantities of very small material.

(3) INCUBATION IN A CLOSED SPACE, WHICH IS NOT SUPPLIED WITH EITHER CAUSTIC OR MANOMETER, WITH OCCASIONAL SAMPLING OF THE AIR FOR ANALYSIS IN SOME FORM OF MICRO-APPARATUS, OF WHICH THE BONNIER-MANGIN APPARATUS IS PERHAPS THE BEST. THIS APPARATUS AND ITS USES ARE DESCRIBED IN DETAIL BY THODAY (*26*). IT HAS BEEN WIDELY USED DURING THE LAST 35 YEARS

In the use of this method seeds have frequently been incubated for respiration in tubes inverted over mercury, but, as pointed out by one of us (*9*), the contact of the seeds with mercury may so alter their behavior as to make the use of this method entirely out of the question in specific cases.

Other methods of incubation without contact with mercury are, of course, possible. But when the mercury is used in contact with the respiring seeds, even if no effect of the mercury is known, this possibility should be carefully investigated before depending upon the results. At best the usefulness of the method is limited by the tediousness of the processes of incubation of the material and of sampling and analysis of the gas.

(4) MICRO-RESPIROMETERS, THE ESSENTIAL FEATURE OF WHICH IS THE USE OF VERY SMALL QUANTITIES OF GAS OR THE DETECTION OF VERY SLIGHT CHANGES IN ITS COMPOSITION

Thunberg's unnecessarily complicated apparatus (*27*), modeled on Peterson's older CO_2 apparatus, consists of two connected pipettes of similar size—one for analysis of the gas and the other for pressure compensation—each connected with a capillary tube and adjustable bulb of mercury. Thunberg's simpler apparatus, merely for demonstration of oxygen consumption (*28*), was modified by Winterstein (*31*), to allow the introduction of artificial atmospheres and further modified by Widmark (*30*) for accurate quantitative work with very small gaseous exchanges. As used by Widmark it is still too cumbersome for general use; one half of the apparatus serves merely as a compensator for the other half, in which the respiring material is incubated, and the results obtained are vitiated by the fact that oxygen consumption and CO_2 production are studied in duplicate lots of material instead of in identical material, or, if in the same material, then in alternate instead of identical periods. Besides, it seems probable that this apparatus could not be used when the gaseous exchange is as large as in most studies with moist seeds.

Tashiro (*26*) described two apparatuses which he claims will detect 1×10^{-7}gr. of CO_2 by absorption in a small drop of barium-hydrate solution. The author claims the possibility of accurate estimation of the amount of CO_2 by a series of trials in any given case. These apparatuses can not be used with as large quantities of CO_2 as are usually involved in respiration studies without extraordinarily large multiplication of experimental errors; they are relatively complicated and expensive; they take no account of the oxygen consumption; and in spite of the author's claim to the contracry, they seem to be adapted only to rather roughly approximate estimation even of the CO_2 given off.

Winkler, long ago, described a very sensitive chemical method for determining the percentage of oxygen in solutions, using alkaline potassium iodid, manganese chlorid, and hydrochloric acid. Recently, Osterhout and Haas (*20*) adapted this method for use in biological work with aquatic organisms. This method is at best too complicated for use when any other method is available. It is not adapted for use with other than aquatic organisms, and it neglects determining the CO_2 produced in respiration.

Warburg (*29*), by substituting a short column of hot *N/80* baryta water for the long column of the cold *N/10*, was able to determine small amounts of respired CO_2 with a maximum experimental error not exceeding 0.1 mgm. or 0.05 cc. CO_2.

Later, Krogh (*14*) developed a manometric apparatus which he described as "the simplest form of the closed-space respiration apparatus." Like Thunberg's apparatus and its various modifications, it makes use of

the principle of the compensating chamber to avoid the necessity of correcting for barometric and temperature changes. It is said to be sensitive enough to follow the oxygen absorption by a single insect egg weighing 2 mgm. in 10-hour periods. Krogh also described a later modification of Winterstein's apparatus which he says can be made even more sensitive than the Krogh apparatus.

Very recently, Lund (*16*) has developed a very simple apparatus for following the CO_2 production by small objects by titration of baryta water. With proper manipulation, it is very sensitive, but, like the other purely chemical methods, Lund's method requires special care to avoid errors due to the absorption of CO_2 from sources other than the respiring material, or errors from other sources. It also neglects oxygen consumption.

The range of some of the apparatuses of the micro type is sufficient to adapt them for much of the work on the respiration of seeds, but none of them provides for simultaneous determination of both sides of the respiratory exchange in the same material.

(5) INDICATOR METHODS

Doctor Haas (*8*) and Professor Osterhout (*17*) recently perfected an indicator method which is very sensitive in detecting small quantities of CO_2 respired. Their method is especially adapted for use in investigations in which only the comparative rate of production of CO_2 under different conditions is to be studied. It has the advantage of sufficient sensitiveness so that the experimental time can be very short, a matter frequently of prime importance; and the small amount of time required for the manipulation of the apparatus adapts the method for rapid and simultaneous determinations in several different experiments. By means of special calibrations and a sampling device the apparatus can be used for the approximate determination of the absolute amounts of oxygen absorbed and CO_2 produced; but this is not its special use. Indicator methods for the determination of the oxygen absorbed by the respiring material have also been described by Osterhout (*18*) and by Harvey (*10*), but these present certain grave objections and at best are not available for simultaneous determination of the CO_2 produced. The use of the indicator method for accurate measurement even of CO_2 output requires constant care to avoid errors due to buffer effects (*19*) or to the possible production of acid-reacting substances other than CO_2 or of alkaline-reacting substances.

In view of the limitations of the apparatuses previously used in investigations of respiration, we undertook to develop a new apparatus which would be simple, easy to use, and free from as many as possible of the objections which other apparatuses presented. With our apparatus, as described on the following pages, oxygen consumption and CO_2 production are determined in the same apparatus and for the same period of time, using the whole volume of air instead of a sample. Both sides of the respiratory exchanges are therefore followed in identical material and without multiplication of experimental errors. The gaseous exchanges are determined at the end of an experimental period by means of a manometer, which is an integral part of the apparatus, somewhat on the plan of Bunzell's oxidase apparatus (*4*). Since the CO_2 given off by the respiring material accumulates and tends to compensate for the oxygen absorbed by it no large changes in pressure occur during any

one experimental period. By making the pressure readings the basis of direct calculations both of oxygen consumed and of CO_2 given off the multiplication of operations and errors involved in a combination of manometric and analytic methods as used by Bonnier and Mangin (*2*) and by Aubert (*1*) is avoided. A number of different forms of apparatus embodying these principles have been constructed and tried, the one here described being finally adopted as giving most satisfactory results.

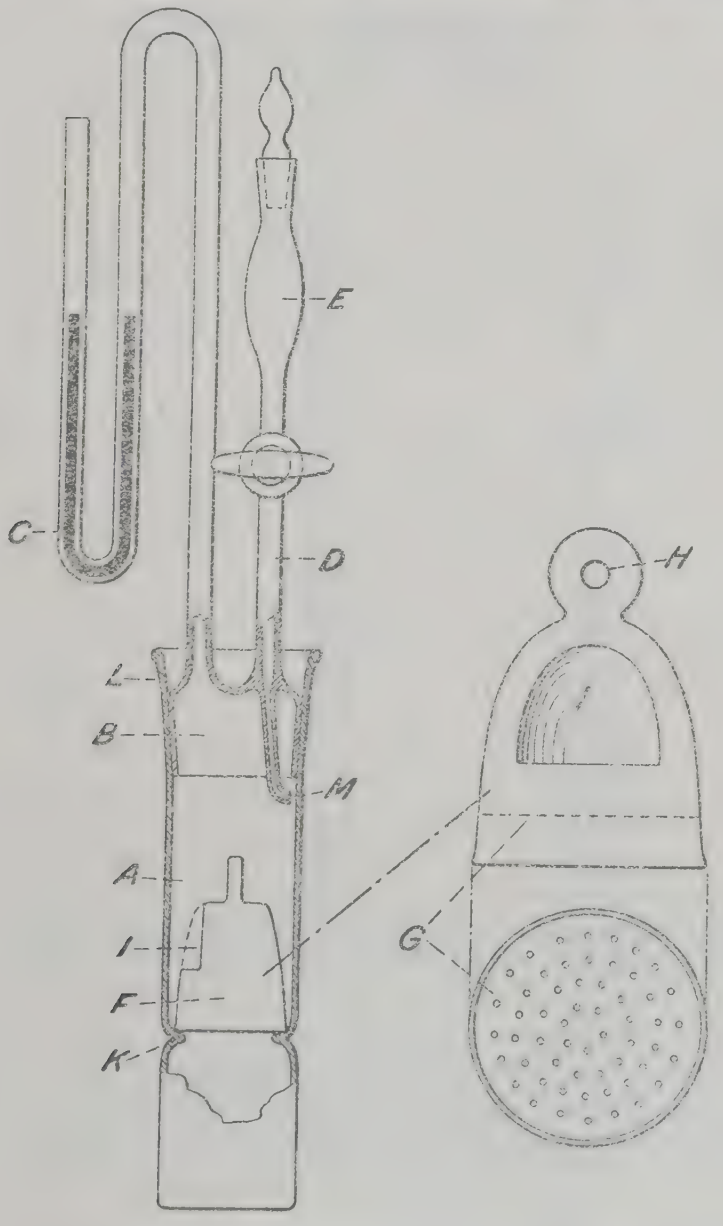

FIG. 1.—Drawing of a respirometer, large size (75 to 80 cc.).

DESCRIPTION OF THE APPARATUS

The apparatus (fig. 1) consists of (1) a cylindrical glass tube (A), in the form of a bottle, which is closed by a ground glass stopper (B), upon which are mounted a short open manometer of 2-mm. bore (C),[3] and an inlet or leveling tube (D) provided with a small chamber (E) for the reception of the caustic which is to be used for the absorption of the CO_2; and (2) a one-piece porcelain container (F) for the seeds.

[3] The bore of the manometers should be at least 2 mm. and may be 2.5, or 3 mm. If the bore is less than 2 mm., the mercury does nor move freely enough to insure accurate work.

This seed container is in the form of an inverted crucible with a plate perforated with about 40 holes 1 mm. in diameter (G) inserted 5 mm. above the open base, and a ring at the top (H) by which, with the use of a bent needle or other suitable hook, to raise it or lower it in the tube (A). In one side of this inverted crucible is an opening (I), reaching from its top to 5 mm. above the perforated plate (G) and covering about 120 angular degrees.

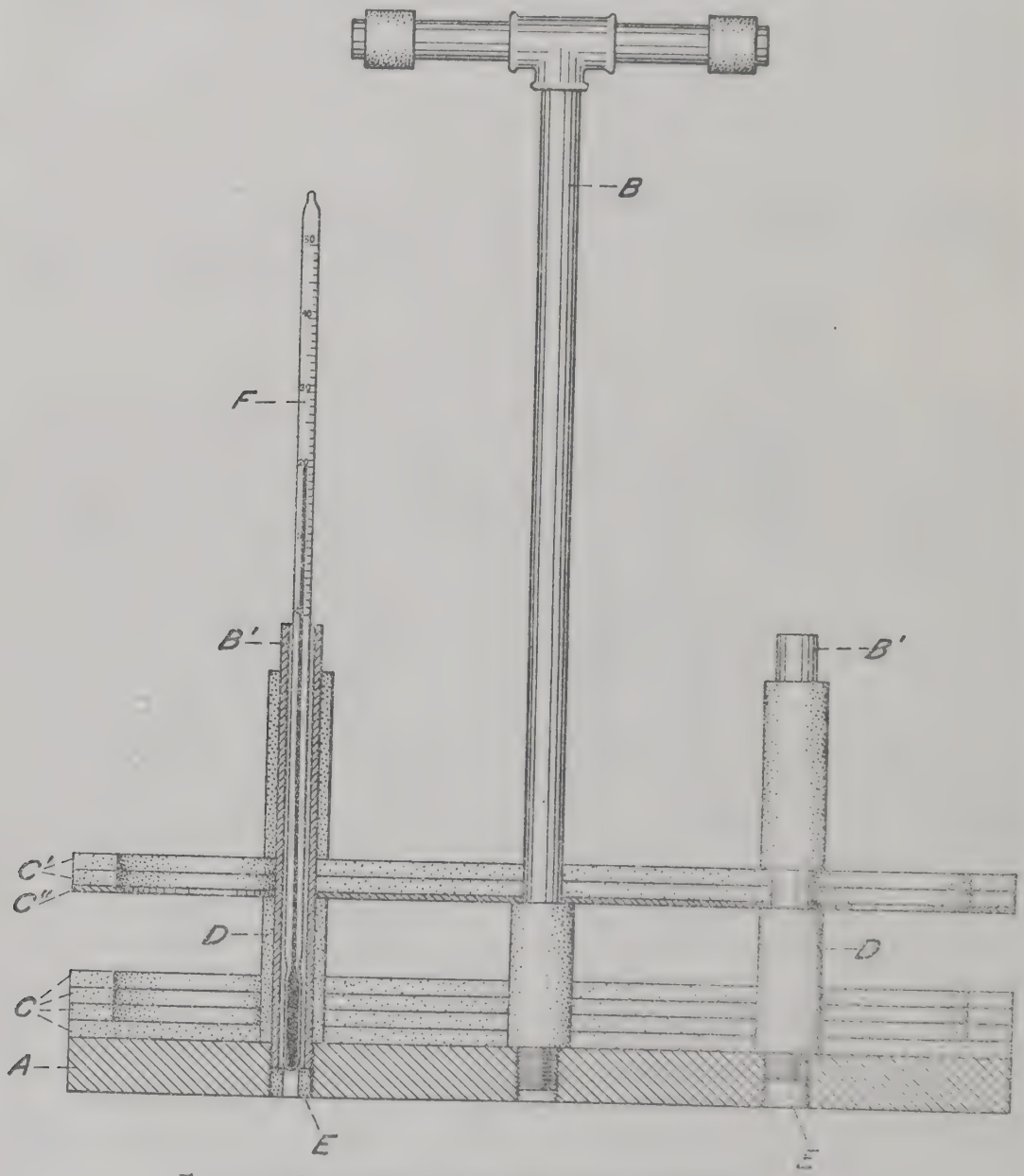

FIG. 2.—Median section of a carrier for eight respirometers.

The seed container rests upon nibs (K) pushed into the sides of the tube (A). The tall, slightly flaring top (L) of this tube provides for reinforcing the ground glass joint by a mercury seal.

We have used this apparatus in three sizes of about 20-cc., 40-cc., and 80-cc. capacity. The tube diameter of all three sizes is 3 cm., so that one size of seed container can be used for all. This simplifies their manufacture and makes them less expensive. With the smallest size, however, the nibs, which support the seed container in the other sizes, are replaced by a very low glass tripod, and the ring at the top of the

seed container has to be omitted. These small apparatuses are so short that the seed containers can be handled by inserting an appropriate hook into the cut-out which receives the seeds, whereas in the larger apparatuses the ring at the top is necessary.

The respiration apparatuses by themselves are hard to handle, so the carrier shown in median section in figure 2 was devised for use with any number up to eight at one time. Into threaded holes bored through a circular cast aluminum base (A) ½ inch thick and 9 inches in diameter are screwed nine posts, which consist of pieces of ¼-inch, heavy-walled brass tubing (B, B′). The tall central post (B) with the T connection serves as a handle, while to the other eight posts, covered with ½-inch thick-walled webb rubber tubing, the eight respiration apparatuses are bound with heavy rubber bands. A number of thicknesses of old rubber matting (C, C′) appropriately placed, and all but the bottom one furnished with eight holes a little larger than the combined sizes of one of the brass posts and a respiration apparatus, hold the respirometers upright and protect them from the breakage which might be caused by bumping on metal.

The upper diaphragm of rubber matting (C′) is stiffened by an underlying diaphragm of sheet aluminum or other appropriate material (C″) and is held at the proper height by short pieces of rubber tubing (D, D′) on the brass posts. The holes in the bottom of the aluminum base are partially closed with small pure gum rubber tubing (E, E) and any one of the eight short hollow posts then serves for the reception of the thermometer (F). In case of fluctuations of temperature, this thermometer should not be depended upon to register the temperature of the water outside of the tubular post in which it is inserted.

OPERATION OF THE APPARATUS

The respiration tubes (fig. 1, A) having been provided with heavy rubber bands, the seed containers with their load of imbibed and sterilized seeds are lowered into place, and the contents of the apparatuses are rapidly aerated by drawing through each a strong current of air. The vasalined ground glass stoppers with the stopcocks opened and the caustic chambers unstoppered are rapidly put in place, forcing each down as far as it will go with a gentle twisting motion and placing them so that the bent delivery tubes (fig. 1, M) from the caustic chambers will deliver onto the closed side of the seed containers. As soon as its top is in place, each apparatus is bound to its post. The whole battery is now submerged in the water bath, so that the water covers the tops of the respiration tubes, and the mercury is poured into these tops. As soon as the whole battery has come to the temperature of the water bath, which may be hastened by gently raising and lowering it, the stopcocks in the leveling tubes are closed, care being taken to see that the respirometers stand vertically and that the mercury is at the same level in the two arms of each manometer, the caustic chambers are stoppered, and the barometric pressure of the room is recorded.

After an appropriate length of time, which will usually be not less than 24 hours, from 1 to 3 cc. as required of 20 per cent sodium hydroxid (NaOH) is measured into each caustic chamber, leaving the stoppers out; the manometers are gently tapped to see that the mercury moves freely to its proper level; manometer readings are taken by holding a scale behind the manometer; the caustic is cautiously admitted into the

respiration tubes; a short interval, during which the barometer may be read, is allowed for complete absorption; and the manometers are again gently tapped and read.

From the manometer and barometer readings, the temperature used in the experiment, and the net volumes of the apparatuses,[4] corrected for the volumes of the respiring material and of the caustic which was admitted, and for the changed positions of the mercury in the manometers,[5] the three significant volumes of air under standard conditions (0° C. at 760-mm. pressure) can be computed. From these three volumes the volume of oxygen absorbed, the volume of CO_2 given off, and the respiratory quotient $\left(\frac{CO_2}{O_2}\right)$ are easily derived.

If extreme accuracy is desired, CO_2-free air may be introduced at the beginning [6] and further corrections may be introduced for the fact that the period for CO_2 production, from the putting of the apparatuses together to the making of the second manometer reading, is slightly longer than that for oxygen consumption, from the closing of the stopcocks to the time of the second manometer reading, and for the further fact that the volume determined for CO_2 respired includes also oxygen absorbed during the very short interval between making the first and second manometer readings.

Since the volume of oxygen absorbed is usually greater than that of CO_2 given off, the reduction of pressure thus brought about in the respirometer starts the flow of caustic when the stopcock is opened, and the absorption of CO_2 also aids in the introduction of the caustic. It is sometimes necessary, however, to force the last of the caustic down by gently blowing into the caustic chamber through a rubber tube slipped over its top.

The caustic, running down the side of the respirometer its entire length and over the top of the seed container, insures rapid absorption of the CO_2. Meantime, the roof of the seed container and the edge which protrudes below its perforated plate protects the seeds completely from contact with the caustic.

After a complete determination the battery of respirometers is removed from the water bath, stopcocks are opened, the mercury seals are removed by suction into a suction flask used as a trap, and the apparatuses are taken apart, emptied, and washed with a dilute acid and then with water. The seed containers may be washed by pouring acid over them without disturbing the seeds, and then both seeds and containers may be further washed under a stream of water by inserting them into Gooch crucibles in such a manner that the open sides of the containers are closed against the sides of the crucibles and the loss of seeds thus prevented.

The containers and seeds are now ready to be returned to the cleaned respirometers for another period of respiration.

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[4] The net air capacity of each apparatus with the seed container in place is determined from the weight of the water it takes to fill it and is corrected for each experiment by subtracting the volumes of the respiring material.

[5] If the bore of the manometer tube is just 2 mm., a change of 3.185 mm. in the height of the mercury on the side toward the respiration chamber—that is, a difference of 6.37 mm. in the height of the mercury in the two arms of the manometer—corresponds to a change of 0.01 c. c. in the uncorrected volume of the air in the apparatus.

[6] The CO_2 in normal air would make a difference of only 0.2 mm. on the manometer, which is not much above the limit of error in adjustment and reading, and would, under the method adopted, affect the values for oxygen consumption and CO_2 production alike. It is believed that leaving a vessel of strong caustic solution exposed to the air for a few hours near where the apparatus is to be set up, and then aerating the apparatus and respiring material with air drawn from the open room over this caustic solution, reduces the error from atmospheric CO_2 well below the lowest possible limits of error. This is the procedure adopted in the work reported in the following article.

These respirometers are adapted for use with small material which does not have to be studied under aquatic conditions, provided it is not markedly sensitive to reduction in the partial pressure of oxygen or to increase in that of CO_2. Some seeds, however, are so small as to pass through the 1-mm. holes of the seed container. For use with such material, the perforations can be partially closed with paraffine. The change in the total pressure of all gases is always small. The manipulations and observations can be performed rapidly and the admittedly somewhat tedious computations may be delayed to suit one's convenience.

The apparatuses could probably be adapted for use with aquatic organisms, at least in certain cases, by substituting an appropriate container for the perforated seed container. They are probably not sufficiently sensitive for use in such work, as, for instance, that of Lund (*15*) with Paramecia, where the total differences in CO_2 production to be measured are sometimes such as would correspond to less than 1-cm. change in pressure as registered on the manometer of the smallest of the apparatuses here described, or about ten time the possible experimental error. In sensitiveness and therefore in the character of work for which they are adapted, they are intermediate between the apparatuses used for large objects and those of the micro type.

Plate 1 is a photograph of the carrier with two respirometers and a thermometer in place. To facilitate the reading of the manometers, which will almost invariably show some reduction in pressure, the respirometers were so constructed that their manometers are turned to the left when the stopcocks in the caustic chambers face outward or away from the tall central post.

The usefulness of the apparatus, as here described, can be greatly extended by attaching to the bottom of the respiration tube a side arm with stopcock for use in the introduction of artificial atmospheres. For work with artificial atmospheres it is desirable also to omit the short delivery tube (fig. 1, M) below the caustic chamber in order to facilitate the complete sweeping out of the original atmosphere. We have a few of the apparatuses in this modified form and have used them with good results, although they embody also some of the less desirable features of our earlier attempts.

The apparatus obviously can not be used except at approximately atmospheric pressure, though the proportions and nature of the gases present can be altered. For the study of respiration at greatly reduced or increased total pressures other forms of apparatus must be used. It will always be true, however, that the great bulk of the work on respiration will be done at atmospheric pressure.

FORMULAE FOR CALCULATION OF THE RESULTS OF RESPIRATION EXPERIMENTS

The following formulae can conveniently be used:

1. For the reduction of volumes to 0° C. and 760-mm. mercury pressure.

$$(1)\quad \text{Vol.}\ {}^{\circ}_{760} = \text{Vol.}\ {}^{t}_{p} \times \left(\frac{273\ P}{760\ (273+t)}\right).$$

$$= \text{Vol.}\ {}^{t}_{p} \times \left(\frac{0.3592\ P}{273+t}\right).$$

in which t and P represent the temperature of the bath in degrees centigrade and the pressure of the gas in millimeters of mercury. The

expression $\frac{0.3592}{273+t}$ represents a factor F which is constant for any given temperature. Substituting F in (1) gives:

$$(2)\quad \text{Vol.}\,{}^{\circ}_{760} = \text{Vol.}\,{}^{t}_{p} \times F \times P.$$

Values for F for different temperatures have been determined and are given here for convenience in calculations.

F for 0° C. = 0.001316
for 5° C. = 0.001292
for 10° C. = 0.001269
for 15° C. = 0.001247
for 20° C. = 0.001226
for 25° C. = 0.001205
for 30° C. = 0.001185
for 35° C. = 0.001166

Values for F for intermediate temperatures can be derived with sufficient accuracy by interpolation in the series given.

2. For changing from gas volumes to milligrams per gram dry weight per 24 hours.

Let a = number of cubic centimeters at 0° C. 760 mm.
b = weight in milligrams of 1 cubic centimeter at 0° C. 760 mm.
c = dry weight in milligrams of respiring material
h = respiration period in hours.

$$(3)\quad \left(\frac{24\ a\ b}{h}\right)\left(\frac{1{,}000}{c}\right) = x = \text{milligrams per gram dry weight in 24 hours.}$$

For oxygen, $b = 1.4289$; for CO_2, $b = 1.9768$.

By substitution of these values and the use of Y in place of one X, equation (3) becomes;

$$(4a)\quad X = \frac{24 \times 1.4289 \times 1{,}000\ a}{c\ h} = \frac{34293.6\ a}{c\ h} \text{ for oxygen.}$$

$$(4b)\quad Y = \frac{24 \times 1.9768 \times 1{,}000\ a}{c\ h} = \frac{47443.2\ a}{c\ h} \text{ for } CO_2.$$

3. For calculating temperature coefficients. Either of the formulae given by Kanitz (*12*) and reported by Denny may be used. Kanitz's second formula is $Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{t_2 - t_1}}$ in which K_2 represents the rate of any reaction at temperature t_2 and K_1 represents the rate of the same reaction at temperature t_1. The easiest form in which to use this equation in logarithmic solutions is

$$(5)\quad \text{Log}\ Q_{10} = (\text{Log}\ K_2 - \text{Log}\ K_1)\left(\frac{10}{t_2 - t_1}\right).$$

With the simplified equations (2), (4a), (4b), and (5) all of the computations can be easily performed.

RECORDING DATA AND CALCULATING RESULTS

Before beginning a respiration experiment the following data must be determined or provision made for its determination:

1. The volume of air in each apparatus without the respiring material (to 0.1 cc.).

2. Volume of the respiring material (to 0.1 cc.).
3. Net volume of air in the apparatus (*1* − *2*).
4. Weight of the respiring material (to 1 mgm.).
5. Percentage dry weight of the respiring material (this to be determined in material duplicating that which is used in the respiration experiment).
6. Dry weight of the respiring material from (*5*) (to 1 mgm.).

All necessary data must be carefully recorded at the beginning and end of each period of a respiration experiment. It is convenient to keep the data for each period in compact form on a 3 by 5 inch card or on a separate sheet of a 3 by 5 inch loose-leaf note book. The following form is suggested:

Exp. begun ..... ; Material .....
No. of period (1, 2, 3, etc.) ..... ; Temp. .....
Stoppers inserted .....
Stopcocks closed .....
Barometer ..... at ..... °C.; = ... at 0°C.
Manometers read ..... ; .....
Caustic used ..... ; Temp. .....
Barometer ..... at ..... °C. = ... at 0°C.
Manometer readings: 1 ..... ; 2 ..... ; 3 ..... ; 4 .....
Before adding caustic .....
After adding caustic .....
Remarks: .....
.....
.....
.....

The absolute net volume of an apparatus uncorrected for pressure or temperature is a base from which all other volumes are calculated, is uniform through any one set of calculations, and therefore need not be determined closer than to tenths of a cubic centimeter. On the contrary, the changes in volume of the gas it contains, corrected to standard conditions, are the actual data for respiratory exchanges. Inaccuracies approaching 0.1 cc. in determining these volume changes would sometimes seriously affect the results of an experiment. All volume computations should therefore be carried to hundredths of a cubic centimeter. For the same reason corrections of apparent volume for the changed positions of the mercury in the manometers should always be made. These corrections will frequently amount to more than 0.1 cc. The caustic solution used should be measured with all possible accuracy and should not be stronger than 20 per cent on account of the impossibility of accurate measurement of a stronger, more viscous solution. As indicated on a previous page, corrections may also be introduced for the short periods of time between the different operations.

Table I illustrates the calculation of gas volumes and units of respiratory exchanges (milligrams oxygen and CO_2 per gram dry weight of the respiring material in 24 hours) from data recorded at the beginning of an experiment and at the beginning and end of one experimental period. The data are taken from the first period of a respiration experiment with duplicate lots of 50 Newtown Pippin apple seeds in a very early stage of germination. The symbols in the left-hand margin of the table are used for convenient reference in later parts of the table to the quantities to which these symbols refer. The formulae, with their numbers, are taken from the preceding section. In practice the form in which these calculations are given can be considerably shortened.

TABLE 1.—*Calculation of gas volumes and units (milligrams per gram dry weight in 24 hours) of respiratory exchange*

Symbol.	Data.	No. 1.	No. 2.
	Volume of empty apparatus	42.1 cc.	44.0 cc.
	Volume of imbibed seeds	3.4	3.4
?	Volume of air in the apparatus	38.7 cc.	40.6 cc.
	Weight of the imbibed seeds	3,052 mgm.	3,112 mgm.
	Percentage of dry weight of the imbibed seeds	53	53
c	Dry weight of the seeds (calculated)	1,618 mgm.	1,649 mgm.
h_1	Stoppers inserted 12.30 p. m., May 27		
h_2	Stopcocks closed 12.45 p. m., May 27		
	Temperature 19.0° C.		
P_1	Atmospheric pressure 762.2 mm. at 22.5° C. =759.3 mm. at 0° C.		
h_3, P_2	Manometer readings 10.00 a. m. May 29	+1.8	+1.3
V_2	Caustic solution added	1.0 cc.	1.0 cc.
h_4, P_3	Manometer readings 10.15 a. m. May 29	−23.5	−23.5
	Temperature 19.0° C.		
P_4	Atmospheric pressure 768.2 mm. at 24° C. =765.0 mm. at 0° C.		
Formula (2)	Vol. $^{\circ}760$ = Vol. $\frac{t}{p}$ F. P.		
	F = 0.001247 − 4/5 (0.001247 − 0.001226) = 0.001230		
Vol. 1	0.001230 V_1P_1	36.14 cc.	37.92 cc.
Vol. 2	.001230 $V_1+\frac{0.01P_2}{6.37}(P_4+P_2)$	36.50 cc.	38.27 cc.
Vol. 3	.001230 $V_1-V_2+\frac{0.01P_3}{6.37}(P_4+P_3)$	34.35 cc.	36.08 cc.
Vol. 4	Approximate volume oxygen = Vol. 1 − Vol. 3	1.79 cc.	1.84 cc.
Vol. 5	Approximate volume CO_2 = Vol. 2 − Vol. 3	2.15 cc.	2.19 cc.
h	Length of period = h_4-h_1 = 45.75 hours.		
	Period for oxygen = h_4-h_2 = 45.50 hours.		
	Period for oxygen included with CO_2. = h_4-h_3 = 0.25 hours.		
Vol. 6	True volume oxygen = Vol. $4+\frac{45.75-45.50}{45.50}$ Vol. 4	1.80 cc.	1.85 cc.
Vol. 7	True volume CO_2 = Vol. $5-\frac{0.25}{45.50}$ Vol. 4	2.14 cc.	2.18 cc.
	Respiratory quotient = $\frac{\text{Vol. 7}}{\text{Vol. 6}}$	1.19	1.18
Formula (4a)	$X=\frac{34293.6a}{c\,h}$; a = Vol. 6	1.80	1.85
	c = Dry weight of seeds	1,618	1,649
	h = 45.75		
	X = mgm. oxygen consumed per gm. dry weight in 24 hours	.83	.84
Formula (4b)	$Y=\frac{47443.2a}{c\,h}$; a = Vol. 7	2.14	2.18
	c = Dry weight seeds	1,618	1,649
	h = 45.75		
	Y = mgm. CO_2 produced per gm. dry weight in 24 hours	1.37	1.36

Table II shows the calculation of temperature coefficients from an experiment with single lots of dormant York Imperial and Newtown Pippin apple seeds in successive periods at 13° and 30° C.

TABLE II.—*Calculations of temperature coefficients from data for successive periods at 13° and 30° C.*

	York Imperial.	Newtown Pippin.
Formula (5) $\text{Log } Q_{10}=(\text{Log } K_2-\text{Log } K_1)\left(\frac{10}{t_2-t_1}\right)$		
K_2 for oxygen consumption (units)	1.25	1.35
K_1 for oxygen consumption (units)	.65	.62
Log k_2	.09691	.13033
Log k_1	9.81291	9.79239
Difference	.28400	.33794
Log difference	9.45332	9.52884
$\text{Log}\left(\frac{10}{t_2-t_1}\right)=\text{Log}\left(\frac{10}{17}\right)$	9.76955	9.76955
Log (log Q_{10})	9.22287	9.29839
Log Q_{10}	.16706	.19879
Q_{10} for oxygen consumption	1.47	1.58
K_2 for CO_2 production (units)	1.47	1.51
K_1 for CO_2 production (units)	.59	.59
Log k_2	.16732	.17898
Log k_1	9.77085	9.77085
Difference	.39647	.40813
Log difference	9.59821	9.61080
$\text{Log}\left(\frac{10}{t_2-t_1}\right)=\text{Log}\left(\frac{10}{17}\right)$	9.76955	9.76955
Log (Log Q_{10})	9.36776	9.38035
Log Q_{10}	.23322	.24008
Q_{10} for CO_2 production	1.71	1.74

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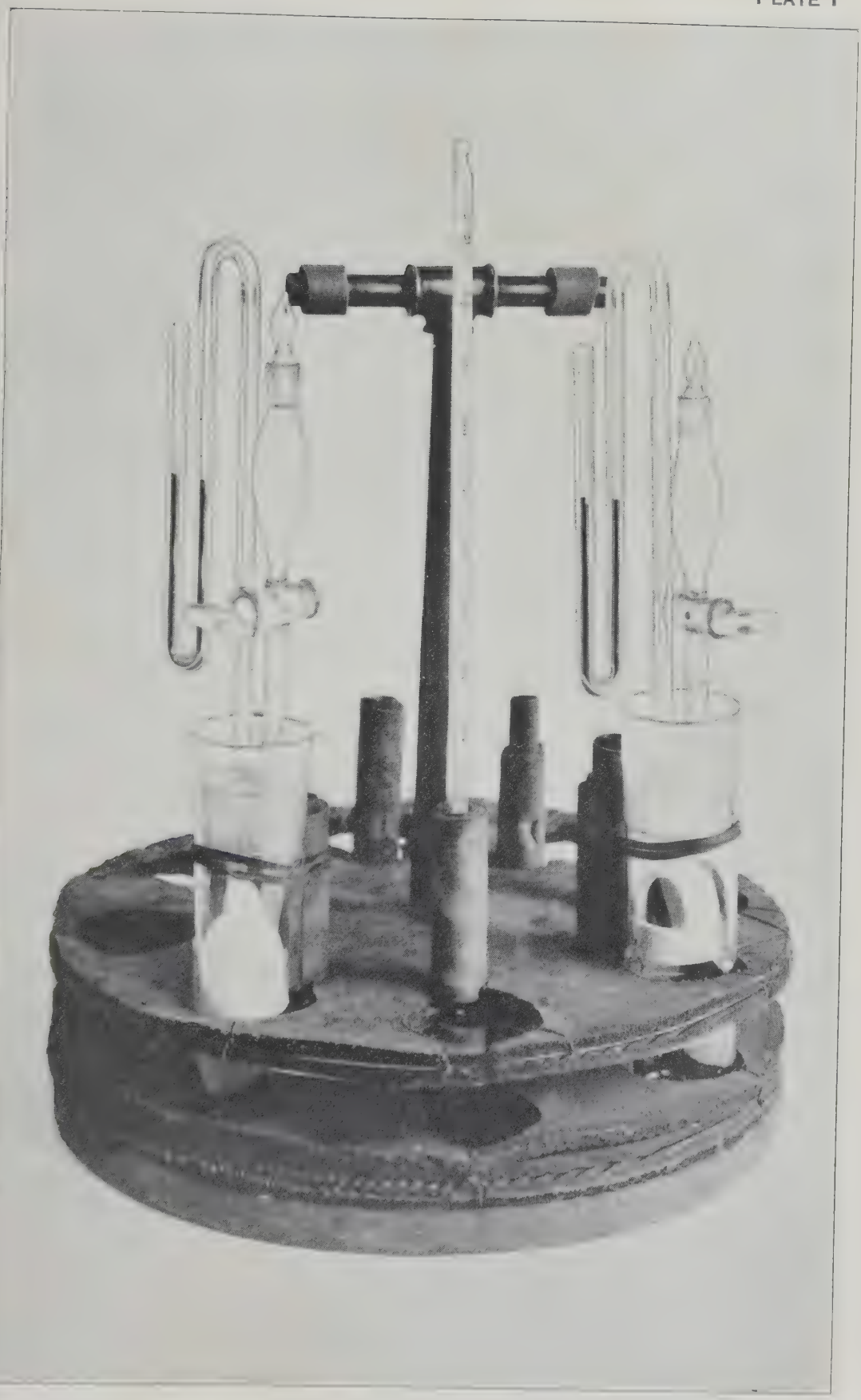
PLATE 1

Photograph of the carrier shown in figure 2, with two large respirometers and a thermometer in place.

PLATE I



RESPIRATION OF APPLE SEEDS[1]

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This study was made, using the manometric respiration apparatuses and following in detail the procedure described in the previous article (*11*) on a new and efficient respirometer for use with seeds and other small objects.

The size of apparatus used in each case was determined by the number and relative activity of the seeds to be studied. The results have been computed in terms of milligrams of oxygen consumed and milligrams of CO_2 given off per gram dry weight of the respiring seeds in 24 hours. The temperature coefficients have been computed for both processes for each temperature interval used, whenever the seeds used were in sufficiently stable condition to make it desirable to do so.

FIRST EXPERIMENT.—Newtown Pippin seeds from fruit stored at 0° C. until May 7. Seeds removed in the next few days and incubated on moist blotters at about 5° to 10° C. Experiment begun May 20 with six parallel lots of seed at temperature of 19°; 25 or 50 seeds per lot at beginning of experiment. (Table I.)

TABLE I.—*Respiration of Newtown pippin seeds at 19° C.*

Period of respiration.	Number of lots.	Condition of seeds.	Gaseous exchanges (mgm. per gm., dry weight) per day.		CO_2/O_2 (volume)
			Oxygen consumed.	CO_2 produced.	
Hours.					
18¼	6	Intact	1.09	1.30	0.86
43	2	Intact; 26 per cent germinating at end of period	.83	1.01	.87
	4	Outer coats removed; 67 per cent germinating at end of period	[a] 2.35 [b] 3.28	[a] 2.73 [b] 3.81	..... .84
21	1	Intact; many germinating at end of period.	.97	1.26	.94
	1	Intact; germinated at beginning of period.	3.42	3.97	.84
	2	Outer coats removed; all germinating at end of period	[a] 2.97 [b] 4.14	[a] 3.15 [b] 4.39	..... .77
	1	Outer coats removed; germinated at beginning of period	[a] 7.48 [b] 10.43	[a] 6.83 [b] 9.53	..... .66

[a] Based on dry weight of intact seeds.
[b] Based on dry weight of seeds with outer coats removed.

[1] Accepted for publication July 2, 1921.

The results after the first period show the effects upon respiration of removing the outer coats [2] and of germination. When the outer coats were removed, the results were computed on the basis of calculated dry weight after removal of these coats as well as on the basis of the dry weight of the intact seeds. Some seeds were discarded during the experiment on account of advancing germination or to leave a convenient number in a given condition for use in a given apparatus.

The respiratory quotient was always less than unity, as should be expected when the material being respired is fatty or oily, and therefore poor in oxygen, as is the case with apple seed.

Removing the outer coats approximately doubled or trebled the respiratory intensity without altering the respiratory quotient until germination had well begun. Connected with this increased respiration of the seeds was an acceleration of germination, though these seeds were sufficiently after-ripened so that the majority of them germinated in four days even in intact condition.

As germination advanced, the respiratory intensity rapidly increased, and in seeds freed from their outer coats the respiratory quotient became lower in value. (See last two entries in last column of Table I.) In intact seeds at a later stage of germination there is a similar decrease in the value of the respiratory quotient, not shown here but illustrated in other experiments.

Godlewski (*9*),[3] Bonnier and Mangin (*4*), Gerber (*8*), and Ivanoff (*13*), have reported low values for the respiratory quotient of germinating oily seeds, or young seedlings.

According to Godlewski, the respiratory quotient of fatty seeds during swelling is a little less than 1.0, but the value of the quotient falls as soon as the roots appear and is maintained about constant at from 0.55 to 0.65 during the period of greatest respiratory intensity, after which it rises to about 1.0. His value for the period of greatest intensity is slightly lower than the lowest shown in Table I. According to this author, and to Gerber, the respiratory quotient of germanating starchy seeds is constantly about unity during the entire period of germination.

Bonnier and Mangin, however, found that with both oily and starchy seeds during germination the respiratory quotient is at first near unity, falls gradually to a minimum variable with the species, and then rises again to its initial value. In this connection, it should be borne in mind that even starchy seeds contain more or less oil. In endospermous, starchy seeds, this oil is usually concentrated in the embryo. If, as would be expected, this oil is drawn upon very early during germination, the large use of oxygen in its conversion into sugars would give a temporarily low value to the respiratory quotient of such seeds, exactly as these authors point out in regard to oily seeds. They conclude that, if there are characteristic differences in the respiratory activity of the two classes of seeds, these differences consist only in the minimum value of the respiratory quotient, and not in the fact or direction of its variation

[2] The coverings of the mature apple seed consist of: (1) a thick brown, fibrous outer coat with open micropyle; (2) a thin, translucent inner coat of very dense structure and without openings, suggesting semipermeable characters; and (3) a delicate, whitish, cellular tissue, somewhat thicker than the inner coat, and evidently endospermous. Layer 3 is closely adherent to layer 2, and it is impossible to remove the two separately, though the embryo is easily bared by removing the two together. The outer coats constituted about 33 per cent, the inner coats and endosperm about 11 per cent, and the embryos about 56 per cent of the wet weight of the seeds. The dry weights of different parts were about 15 per cent, 5 per cent, and 33 per cent of the original wet weight of the whole seeds.

Reference is made by number (italic) to "Literature cited," p. 129–130.

Gerber gives a minimum value of about 0.30 for the respiratory quotient of germinating oily seeds, while Ivanoff reports values as low as 0.13. Gerber argues from his results that if the particular fats stored in any given seed are easily oxidized (flax) the respiratory quotient will be near that for complete oxidation (0.70), while if they are difficultly oxidizable (Ricinus) the quotient will be nearer that for simple conversion of the fat to sugars and therefore relatively low.

Table I also shows a tendency for the value of the respiratory quotient to rise temporarily in the initial stages of germination of intact apple seeds. Gerber (*8*) points out a similar condition when the radicles are unable to break the coats, and assumes, without proper evidence, that his occasional high values of the respiratory quotient (slightly greater than 1.0) in such cases are due to the transformation of sugars into alcohol in the "imprisoned and fatigued" cells. The same result would be obtained by the splitting up of the carboxyl groups of organic acids—a reaction, which, like the formation of alcohol, might result from a deficiency of oxygen in the respiring tissue. This oxygen deficiency, in turn, might occur as a result of the limiting effects of coat structures relatively impermeable to oxygen. But it is also true that a rise in the respiratory quotient until it becomes nearly equal to 1 might result from the oxidation of previously accumulated sugars more rapidly than the fats are broken down, without any reference to oxygen deficiency. Irwin (*12*) also calls attention to the theoretical possibility of obtaining a high production of CO_2 as a result of the splitting of carbonates and bicarbonates in the respiring tissues by accumulating organic acids. Since free oxygen would ordinarily be consumed in the formation of the organic acids, this oxygen consumption would tend to balance the CO_2 produced by such a breaking up the carbonates and to hold the respiratory quotient at about its previous value. In Irwin's work the increased production of CO_2 by Salvia petals as a result of etherization was accompanied by decreased acidity, and a simultaneous increase in oxygen consumption indicated that the increased CO_2 production resulted from true respiratory exchanges. It is regrettable that Irwin's apparatus (the Haas-Osterhout indicator apparatus (*19*) and technic did not involve the simultaneous determination of oxygen consumption and CO_2 production so that the respiratory quotient could be determined and closer deduction could be drawn as to the probable origin of the increased output of CO_2. In the absence of such data, it is natural to suppose that reduction in acidity at the same time that CO_2 production was increased would involve an oxidation of organic acids and an increase in the respiratory quotient.

SECOND EXPERIMENT.—Seeds from a cider press mash, not germinated after incubation as follows:

Lot A, 50 days at 20° C. in intact condition, then 68 days at 20° with outer coats removed.[4]

Lot B, 50 days at 30° C. in intact condition, then 68 days at 20 to 30°, daily alternation, with outer coats removed.[4]

Lot C, 50 days at 20° C. in intact condition, then 68 days in an ice box at about 5° to 10°, with outer coats removed.[4]

The experiment was begun May 27, 1919, 25 seeds in each lot.

All the seeds in lot C germinated within the first two periods of two days each at 19° C. During the first period the volume of gaseous ex-

[4] See footnote 2 on page 118.

changes was about twice and during the second period about four times that for lots A and B, which remained dormant. The respiratory quotients for the two periods were 0.81 and 0.75—a little higher than for lots A and B.

Table II shows the respiratory activity of lots A and B at different temperatures during a period of two months. During this time 12 per cent of the seeds germinated and were discarded, while a few others were discarded on account of injury. All results were calculated on the basis of the dry weight of the material actually present in the apparatus whether this included the inner coats or these had been removed.

Both the respiratory intensity and the respiratory quotient rose and fell as the temperature of the experiment was raised and lowered. For each temperature, there was a fairly definite respiratory quotient which was reestablished each time the seeds were brought back to that temperature from another temperature, either higher or lower.

TABLE II.—*Respiration of dormant cider press apple seeds with outer coats removed*

Period	Temperature.	Gaseous exchanges (milligrams per gram dry weight per day).				CO_2/O_2 (volume)	
		Oxygen consumed.		CO_2 produced.			
		A [1]	B [2]	A [1]	B [2]	A [1]	B [2]
Hours	°C						
45	19	1.02	1.35	0.98	1.19	0.69	0.64
46	19	1.00	1.61	.99	1.63	.72	.73
46	19	1.33	1.61	1.36	1.52	.74	.69
21	30	2.97	2.94	3.23	3.07	.79	.75
46	30	3.20	2.56	3.39	2.44	.77	.69
45	30	4.23	2.93	4.73	3.03	.81	.75
43	19	2.86	2.83	2.61	2.64	.67	.67
45	19	2.80	2.53	2.57	2.24	.66	.64
47	19	2.77	1.39	2.48	1.25	.65	.65
25	30	5.59	2.05	5.67	1.74	.73	.61
48	30	4.40	1.95	4.70	1.85	.77	.68
48	19	3.25	1.81	3.00	1.69	.67	.67
118	19	3.31	1.28	3.12	1.16	.68	.65
115	10	1.60	.98	1.37	.76	.62	.56
121	10	1.64	1.06	1.40	.86	.62	.59
118	10	1.55	.97	1.35	.77	.63	.58
194	0	.43	.38	.12	.21	.21	.40
140	13	1.81	1.07	1.72	.95	.69	.64
49	30	4.47	3.30	4.91	3.27	.79	.72
INNER COATS REMOVED.[3] INCIPIENT GERMINATIONS BY END OF PERIOD							
20	30	19.45	16.74	22.91	17.87	.85	.77
ALL GERMINATING BY END OF PERIOD							
43	19	10.09	8.86	10.30	9.61	.74	.78

[1] Lot A was previously incubated under germination conditions at 20° C., 50 days in intact condition, then 68 days with outer coats removed.

[2] Lot B was previously incubated under germination conditions, 50 days at 30° C in intact condition, then 68 days t 20 to 0° C with outer coats removed.

[3] See footnote 2 on page 118.

Lot B showed a generally lower respiratory intensity, lower respiratory quotients, and a smaller change in respiratory quotient resulting from any given change in temperature than did lot A. All of these differences seem to indicate that lot B was in a state of deeper dormancy than lot A.

This condition probably reflects a greater impoverishment of lot B in the materials used in respiration as a result of previous incubation at a higher temperature. Both the greater intensity of respiration and the higher respiratory quotient at the higher temperature would work in this direction.

This conception of the relation of dormancy to respiratory exchanges follows closely the observations of Lund and others. Lund (*16*) has shown a great reduction in respiratory intensity of Paramecia as a result of partial starvation, or, in other words, impoverishment in respirable materials. The character of his material would naturally lead one to expect a much greater reduction in respiratory intensity on account of such impoverishment than would occur in dormant seeds, whose respiration is already on a rather low plane. In a later paper (*17*) the same author has shown that loss of irritability by Planaria under anaerobic conditions is much more closely associated with decreased CO_2 production than with absence of consumption of free oxygen. In the seeds of lot B, a decrease in CO_2 production relative to oxygen consumption under aerobic conditions seems likewise to correspond to a state of greater quiescence or deeper dormancy.

Lot A increased in intensity of respiration at a given temperature, perhaps as a result of the temperature changes. Lot B, which had previously been given a regular daily alternation of temperatures, showed a greater intensity of respiration than lot A during the first few periods of the experiment, increased temporarily, and then fell to the original intensity. The effect of alternating temperatures upon the respiration of dormant seeds is, however, unknown, and may bear no relation to the differences here noted.

Ziegenbein (*23*), working with germinated seeds, decided that sudden changes of temperature cause no modification of respiratory intensity at either extreme of the alternation. On the other hand Palladine (*20*), using similar material which had been previously cultivated on sugar solutions at different temperatures, concluded that passing from a high temperature (36° C) or from a low temperature (7°) to a medium temperature (18° to 20°) stimulates the respiration of plants. Blanc (*3*) objected to Palladine's technic and, in a very careful piece of work upheld Ziegenbein's view. Blanc concluded that for each temperature there is a definite respiratory intensity, and the transition from the respiratory activity corresponding to a given temperature to that corresponding to a different temperature is made gradually allowing for all respiratory activities intermediate between those of the extreme temperatures.

While Blanc's results seem conclusive for the material he worked with, neither he nor the previous workers used material which was in a dormant condition. In view of the known effect of temperature alternations upon the germination of many kinds of seeds it seems possible that the respiration of dormant seeds may at least in some cases be affected by such alternations in ways quite different from any effect upon actively growing material. This point needs investigation. My own results as here given, while suggestive, certainly furnish no proof of such effects.

The very low respiratory quotient at 0° C. is particularly interesting because it suggests storage of oxygen at low temperatures as at least one cause of the favorable effect of low temperatures upon after-ripening. Even at 19°, the weight of oxygen taken in was greater than that of CO_2 given off, so that unless some of the excess oxygen was split off as water, the seeds were actually gaining in dry weight. The obvious suggestion here is that oxygen is being used in the transformation of fats to sugars or in the formation of organic acids. Sugars and acids have been shown by Applemann (*1*), Atwood (*2*), Brannon (*5*), Eckerson (*7*), and Jones (*14*) to accumulate during storage of dormant structures at low temperatures. Eckerson (*7*), in fact, showed an increase in acidity of haw and apple seeds during incubation at low temperatures, which were favorable for after-ripening. I have found that the after-ripening of apple seeds takes place also in commercial cold storage when the seeds remain within the intact fruit. While perhaps it should not be expected in this case that oxygen would be available for storage in the seeds, still the volume of the respiratory exchanges of the seeds within the fruit must be small and must be governed more or less by the respiratory activity of the surrounding pulp, so that the maintenance of a low respiratory quotient, with concurrent increase in the acidity of the seeds, may not be out of the question even here.

Possibly also the increase of oxidizing enzyms during after-ripening at low temperatures, as shown by Eckerson for apple seeds, and by Crocker and Harrington (*6*), Jones (*14*), and Rose (*21*) for other seeds, and the fact that their catalase activity at least is exceedingly high immediately after germination, as I have found in work as yet unpublished, and as previously reported by Crocker and Harrington (*6*) and by Jones (*14*) for other seeds, are significantly related to their respiratory exchanges, especially to storage of oxygen and low respiratory quotient during after-ripening at low temperatures and to intense oxidation with high respiratory quotient in the first stages of germination. The correspondence between respiratory activity and catalase activity is especially interesting in view of previous work by Applemann (*1*), Crocker and Harrington (*6*), and Kohl (*15*) which seems to connect this enzym definitely with respiratory processes.

In the last two periods of the experiment, the naked embryos were used. Germination began at once, and in less than three days all had germinated There was the customary tremendous increase in respiratory intensity. There was also a slight increase in the respiratory quotients above those which were normal for the dormant seeds at the temperatures used, indicating that, in this early stage of germination, oxygen-rich substances were being respired more rapidly than the fats were being broken down to their component sugars. During the last period the oxygen in the respiration apparatuses, as computed from their known volume, was quantitatively used up, and the respiratory quotients were probably somewhat higher than they would otherwise have been.

The temperature coefficients for oxygen consumption and for CO_2 production in consecutive periods of the preceeding experiment are given in Table III.

Table III shows values for Q_{10} ranging from scarcely more than 1 to nearly 4, with two exceptional higher values for CO_2 production at low temperatures in case of lot A. The values are greater for lot A than for lot B, emphasizing again the difference in response of the two lots to

external conditions. They are also greater for CO_2 production than for oxygen consumption, greater when changing from 19° to 30° C., than when the reverse change is made, and greater at low temperatures than at higher temperatures. The differences in temperature coefficients, when the reciprocal changes 19° to 30° and 30° to 19° are made, suggest a stimulating effect of the higher temperature, which is not entirely lost upon return to the lower temperature.

Upon removal of the inner coats and the onset of rapid germination, with vigorous respiration, the temperature coefficients remained about the same as for the same temperature interval with dormant seeds.

TABLE III.—*Temperature coefficients (Q_{10}) for the respiration of dormant apple seeds with outer coats removed*

Temperature interval.	Q_{10} for oxygen consumption.		Q_{10} for CO_2 production.	
°C.	A[1]	B[2]	A[1]	B[2]
19 to 30	2.08	1.73	2.20	1.90
30 to 19	1.43	1.03	1.71	1.14
19 to 30	1.89	1.43	2.12	1.35
30 to 19	1.32	1.07	1.51	1.09
19 to 10	2.32	1.35	2.46	1.53
10 to 0	3.63	2.53	11.04	3.60
0 to 13	3.03	2.21	7.66	3.15
13 to 30	1.70	1.59	1.85	2.07
30 to 19 naked germinating embryos	1.95	1.78	2.07	1.76

[1] Lot A was previously incubated under germination conditions at 20°C., 50 days in intact condition, then 68 days with outer coats removed.
[2] Lot B was previously incubated under germination conditions, 50 days at 30°C. in intact condition, then 68 days at 20° to 30° with outer coats removed.

THIRD EXPERIMENT.—Newtown Pippin seeds. Fruit stored at 0° C. to May 7, seeds removed during next few days and incubated under germination conditions at 5° to 10° for 13 to 18 days. Many seeds had germinated. Only those not germinated were used for the respiration experiment. Fifty seeds in each lot at beginning of experiment. Some discarded from time to time on account of advancing germination or to keep a convenient number in a given condition for a given apparatus. (Table IV.)

The principal points to be noted regarding this experiment are:

1. The respiratory intensity which was about the same at the beginning of the experiment as shown in Table I, rose sharply with the onset of germination, but fell somewhat after germination, with the second period at the excessively high temperature of 30° C.

2. The respiratory quotient was high, as in the previous experiments, during incipient germination. The value of the respiratory quotient in this case reached 1.2, recalling Gerber's observations.[5]

3. The respiratory quotient fell in value as respiratory intensity increased with advancing germination. The lowest value of the respiratory quotient, as well as the figures expressing respiratory intensity during the same period, are here almost identical with those for the period of greatest respiratory intensity in Table I.

[5] See discussion on page 119

TABLE IV. *Respiration of Newtown Pippin seeds from cold-stored fruit. Seeds not germinated after incubation at 5° to 10° C. for 13 to 18 days*

Period.	Temperature.	Description of seeds.	Gaseous exchanges. (Mgm. per gm. dry weight per day.)		CO_2/O_2 (volume).
			Oxygen consumed.	CO_2 produced.	
Hours.	° *C.*				
45	19	Several incipient germinations at end of period.	0.95	1.58	1.20
		Duplicate lots	.95	1.56	1.19
46	19	54 per cent germinating by end of period. Roots reddish.	.97	1.28	.95
46	19	A. Not germinated; 50 per cent germinating by end of period.	1.49	1.70	.83
		B. Germinating at beginning of period	1.94	2.19	.81
21	30	A. Not germinated; 44 per cent germinating by end of period.	1.80	2.78	1.11
		B. Germinated at beginning of period; slow growth.	11.15	10.00	.65
46	30	A. Same seeds as previous period; 52 per cent germinating by end of period.	2.01	2.56	.92
		B. Same seeds as previous period	6.13	5.97	.70

FOURTH EXPERIMENT.—Newtown Pippins. Cold stored in fruit until May 7. Seeds removed and incubated a few days at 5° to 10° C., then 12 days at 25°. Many germinated at 25°. Respiration experiment with those not yet germinated. Twenty-five seeds in each lot at first. Some discarded during the experiment on account of germination or to leave a convenient number in a given condition for a given apparatus. Experiment begun May 27, 1919. (Table V.)

The principal points to be noticed in this experiment are:

1. Respiratory intensity was lower during the first period than in the previous experiment with similar seeds previously incubated only in the ice box. This difference in resipratory intensity between the seeds used in the two experiments is correlated on the one hand with a possilbe assumption of a condition of secondary dormancy by the seeds at 25° C., and on the other hand with a known increase in metabolic activity during the last stages of after-ripening at the lower temperature. To be sure the selective effect of germination during the previous incubation would leave only the less active seeds to be used in the respiration experiments. But this factor could not account for the difference observed. Of the seeds originally put to germinate, nearly twice as large a percentage of those incubated at 5° to 10°, had germinated previous to the respiration experiment as was the case with those incubated at 25°. The selection resulting from germination would, therefore, of itself tend toward a lower rather than a higher respiratory activity in the seeds used in the previous experiment as compared with those used in the present experiment. Since the two experiments were begun at the same time, and conducted simultaneously throughout, there can be no difference due primarily to the mere lapse of time.

TABLE V.—*Respiration of Newtown Pippin seeds, not germinated after cold storage in the fruit to May 7, incubation at 5° to 10° C. for a few days and at 25° for 12 days*

Period.	Temperature.	Condition of seeds.	Gaseous exchanges. (mgm. per gm., dry weight, per day.)		CO_2/O_2 (volume).
			Oxygen consumed.	CO_2 produced.	
Hours.	° *C.*				
45	19	Dormant	0. 80	0. 77	0. 70
46	19	A. 12 per cent just beginning to germinate at end of period	. 85	. 83	. 72
		B. 24 per cent germinating at end of period; longest root 6 mm	1. 45	1. 42	. 71
46	19	A. Not germinated; 16 per cent germinating at end of period	1. 82	2. 25	. 89
		B. All germinated at beginning of period; fairly active growth; healthy	7. 54	6. 62	. 63
21	30	A. Not germinated; 10 per cent germinating at end of period	1. 45	1. 71	. 85
		B. Same seeds as B in preceding period; little new growth; roots curled and reddish	13. 4	11. 3	. 61
46	30	A. Same seeds as in preceding period; 15 per cent germinating and 5 per cent moldy by end of period	1. 36	1. 53	. 81
		B. Same seeds as B in preceding period; growth slow	9. 69	8. 55	. 64

Furthermore, contemporaneous germination tests showed that very little germination occurred at 25° C. after the first 12 days, while at 5° to 10°, or a little above, germination continued slowly and was nearly complete within the next two weeks. There was therefore a progressive change going on in each case, tending to a condition of dormancy at 25°, and to complete germination at the lower temperature. The impoverishment of the seeds used in this experiment as a result of relatively high respiratory intensity during the previous period at 25°, and especially the relatively high respiratory quotient no doubt obtaining at that temperature on the one hand, and the low respiratory intensity and storage of oxygen (low respiratory quotient) characteristic of the lower temperature on the other hand, are no doubt related to the comparative behavior, both as to respiration and as to germination, when the seeds used in the two experiments were brought to the same intermediate temperature of 19°.

2. The respiratory intensity increased rapidly with advancing germination, but fell off during the second period at the excessively high temperature of 30° C.

3. The respiratory quotient corresponded to complete oxidation of the fats (0.70) at the beginning of the experiment, rose very slightly during the earliest emergence of radicles and much more during later incipient germinations, and fell markedly in later stages of germination in spite of the high temperature (30° C.), which gives a high respiratory quotient in dormant seeds.

FIFTH EXPERIMENT.—A. Seeds remaining in lot A of previous experiment of which this experiment is a continuation; B, 25 York Imperial seeds not germinated after cold storage in the fruit to February 5, 1919, removed from fruit February 15, and incubation at 5° to 10° C., four days, and at 25° 3½ months. This half of the experiment was begun May 27, 1919. (Table VI.)

TABLE VI.—*Respiration of Newtown Pippin and York Imperial seeds dormant after cold storage in the fruit and incubation at 25° C.*

Period.	Temperature.	Gaseous exchanges (mgm. per gm. dry weight) per day.				CO_2/O_2 (volume).	
		Oxygen consumed.		CO_2 produced.		A[1]	B[2]
		A[1]	B[2]	A[1]	B[2]		
Hours.	° *C.*						
45	19	.....	0.80	.....	0.81	.....	0.73
46	19	.....	.93	.....	.91	.....	.71
46	19	.....	.97	.....	.94	.....	.71
21	30	.....	1.56	.....	1.80	.....	.83
46	30	.....	1.19	.....	1.18	.....	.72
45	30	1.36	1.11	1.27	1.03	0.68	.67
43	19	.96	1.01	.78	.78	.59	.56
45	19	.85	.90	.91	.80	.77	.64
47	19	.81	.84	.86	.69	.77	.59
25	30	1.57	1.33	1.59	1.25	.73	.68
48	30	1.06	1.00	1.02	1.02	.81	.74
48	19	.93	.94	.90	.78	.70	.60
118	19	.59	.63	.59	.57	.72	.65
114	10	.60	.61	.51	.49	.61	.57
121	10	.62	.64	.51	.54	.59	.58
118	10	.62	.65	53	.46	.62	.50
194	0	.28	.30	.14	.16	.37	.39
140	13	.62	.65	.59	.59	.70	.66
49	30	1.35	1.25	1.51	1.47	.81	.85
			ALL OUTER COATS REMOVED[3]				
20	30	5.22	4.54	5.05	4.14	.70	.66
INNER COATS REMOVED[3]			ALL GERMINATING BY END OF PERIOD				
43	19	9.56	7.19	9.53	6.99	.72	.70

[1] Lot A, Newtown Pippin seeds dormant after cold storage in fruit, incubation at 5° to 10° C for a few days and then 25° for 12 days, and previous respiration experiment at 19° and 30° for 9 days.

[2] Lot B, York Imperial seeds dormant after cold storage in fruit, incubation at 5° to 10 ° C. for 4 days and at 25° for 3½ months.

[3] See footnote on page 118.

Table VI gives the data for the respiratory exchanges. None of these seeds germinated until the last period of the experiment after removal of the inner coats. All results were calculated on the basis of the dry weight of the material used in each period, whether this included coat structures or not.

1. The respiratory intensity was generally much lower than that of the dormant seeds with outer coats removed, which were used in experiment 2, but on account of the method used of computing the results in relation to the material actually used, the differences in relation to the actively respiring portions were probably not as large as would appear from a comparison of the two tables.

During the last long period (118 hours) at 19° C. the respiratory intensity was so reduced that for the whole period the average intensity was about the same as for the subsequent periods at 10° and for a later period at 13°. It is not possible to state the reason for such a fall in respiratory intensity during this period. It can not be the direct result of greater reduction in oxygen pressure or greater increase in CO_2 pressure than in previous periods, since the actual amounts of gaseous

exchange were scarcely greater during this period than during some previous periods at 30° C.

2. Respiratory quotients were generally about the same as in experiment 2, but rather irregular, especially with lot A in the first few periods following the preceding experiment in which many germinated; except for these irregularities quotients rose and fell with rise and fall of temperature, and they became very low at 0° C.; the respiratory quotients were higher for lot A than for lot B, probably for the reason suggested in experiment 2 (see page 120).

3. Removal of the outer coats greatly increased respiratory intensity but decreased respiratory quotients as if rendering oxygen more available. If this is the correct explanation, then it must be that the inner coats are readily permeable to oxygen in aqueous solution, and this must hold, too, for CO_2. Such an assumption would explain the greater respiratory intensity of the seeds used in experiment 2, as compared with those used in experiment 5, and the sporadic germination of a small percentage of the seeds used in experiment 2 during the course of the experiment would then appear as probably the result of such increase in permeability and consequent increase in respiratory intensity. With prolonged exposure of the inner coats to the air, as in the case of experiment 2, they become somewhat brownish and perhaps less permeable.

4. With removal of the inner coats, the seeds all germinated with greatly increased respiratory intensity and little change in respiratory quotients. The air in the apparatuses at the end of this period contained about 1 per cent oxygen.

Table VII gives the temperature coefficients for oxygen consumption and CO_2 production for consecutive periods with all temperature intervals used in experiment 5.

TABLE VII.—*Temperature coefficients for respiration of dormant intact apple seeds*

Temperature interval.	Q_{10} for oxygen consumpton.		Q_{10} for CO_2 production.	
	A [1]	B [2]	A [1]	B [2]
°C.				
19 to 30	.....	1.54	.....	1.80
30 to 19	1.37	1.09	1.56	1.29
19 to 30	1.83	1.52	1.75	1.71
30 to 19	1.13	1.05	1.30	1.28
19 to 10	.99	1.02	1.18	1.19
10 to 0	2.25	2.17	3.79	2.79
0 to 13	1.86	1.80	3.04	2.69
13 to 30	1.59	1.48	1.73	1.71

[1] Lot A. Newtown Pippin seeds dormant after cold storage in fruit, incubation at 5° to 10° C. for a few days and then 25° for 12 days, and previous respiration experiment at 19° and 30° for 9 days.

[2] Lot B. York Imperial seeds dormant after cold storage in fruit, incubation at 5° to 10° C. for 4 days and at 25° for 3½ months.

As in experiment 2, the values for Q_{10} are greater for CO_2 production than for oxygen consumption, greater for the lot which I have supposed to be richer in the more easily oxidizable materials (A) than for the other lot (B), greater when changing from 19° to 30° C. than when the reverse change was made, and greater at low temperatures than at higher temperatures.

As a result of the very low respiratory intensity during the 118 hour period at 19° C., the temperature coefficients from this temperature to 10° appeared very low.

From Table VII and the previous table of temperature coefficients (Table III) it is evident that the values of Q_{10} for the respiration of seeds for any given temperature interval may depend upon a number of different factors, which have not as yet been worked out, but among which the physiological condition of the seed and its previous treatment are important.

It is impossible to draw reliable conclusions as to the nature of the complex of processes which constitute respiration from any one set of temperature coefficients. Nevertheless, it seems entirely safe to say that different steps in the complex are differently affected by temperature changes—in other words, have differential temperature coefficients—and that these differences are reflected in the physiological condition of the living embryo can scarcely be doubted.

IMPORTANCE OF STUDYING OXYGEN CONSUMPTION AND CO_2 PRODUCTION IN THEIR RELATION TO EACH OTHER

A great many, though by no means all, investigators of respiration have confined their attention to either oxygen consumption or CO_2 production to the exclusion of the other, and frequently the apparatus used has rendered the study of the other impossible. Different investigators have been led to consider of paramount importance one or the other of these two evidences of respiratory activity. Mayer (*18*), for instance, studied only oxygen consumption because this was supposed to correspond much more nearly to the amount of heat produced by respiration than does CO_2 production. Much of the oxygen consumed does not reappear as CO_2 but is built into the plant tissues. In this case CO_2 is not the end product of the oxidation. On the other hand, he points out that CO_2 is given off by the reduction of oxygen-rich mineral acids, quite independent of true respiration. Tashiro (*22*) and, following him, Gurjar (*10*) emphasized CO_2 production as "an accurate index of respiratory activity."

It would seem from the data in this paper that neither oxygen consumption nor CO_2 production can be considered as "an accurate index of respiratory activity." Both depend upon external as well as internal conditions, which affect the two differently, so that neither alone gives a complete picture, much less a satisfactory understanding of respiratory exchanges. It seems certain, furthermore, that following oxygen intake and CO_2 output, in their relation to each other and to external factors, will be useful in pointing the way to fruitful investigations in other fields of plant physiology.

SUMMARY

(1) The respiratory intensity of dormant apple seeds is low. The respiratory intensity of seeds capable of germination is higher and becomes very high with advancing germination but soon falls somewhat if the germinated seeds are kept at too high a temperature (30°).

(2) Removal of the outer seed coats or of both seed coats increases respiratory intensity and accelerates germination.

(3) The respiratory quotient of dormant apple seeds at ordinary temperatures (19° C.) corresponds to complete oxidation of fats (0.70) or to only slight increase in sugars.

(4) The respiratory quotient increases with increase in temperature, causing impoverishment in easily oxidizable substances and possibly indicating oxygen deficiency in the respiring tissues.

(5) The respiratory quotient decreases with decrease in temperature, indicating a storage of oxygen which becomes very considerable at 10° and 0° C. and probably leads to increase in acids and sugars.

(6) Correlated with a relatively high rate of oxidation at high temperatures is a tendency for the seeds to become more dormant; with storage of oxygen at low temperatures they slowly become capable of germination.

(7) The respiratory quotient becomes low (about 0.60) with advancing germination, indicating the rapid transformation of fats and accumulation of sugars. Preceding this fall in respiratory quotient there is frequently a brief initial rise (to a maximum of 1.2), which indicates the breaking up of oxygen-rich bodies (probably organic acids) much more rapidly than these bodies are replaced and may be associated with a temporary deficiency of oxygen in the respiring tissues.

(8) The temperature coefficients for the respiratory exchanges of dormant apple seeds are greater for seeds previously incubated at medium temperatures (20° C.) than for seeds previously incubated at higher temperatures, greater for CO_2 production than for oxygen consumption, greater when changing from a medium temperature (19° C.) to a higher temperature (30° C.) than when the reverse change is made, and greater at low temperatures than at high temperatures.

(9) Temperature changes, at least temporary elevation of the temperature (to 30° C.), may exert a stimulating effect on the respiration of dormant apple seeds, but the evidence is not conclusive.

(10) Respiratory intensity, respiratory quotients, and temperature coefficients are affected by the previous treatment of the seeds, being higher after treatment which tends toward after-ripening, and lower after treatment which induces deeper dormancy.

(11) Temperature coefficients are different for different steps in the oxidative processes which constitute respiration, and these differences are related to the different temperature effects upon the physiological condition of the living embryo.

(12) In order to gain an understanding of respiratory processes it is necessary to study oxygen consumption and CO_2 production in their relation to each other.

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TWO IMPORTANT NEW TYPES OF CITROUS HYBRIDS FOR THE HOME GARDEN—CITRANGEQUATS AND LIMEQUATS[1]

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The breeding of cold-resistant citrous fruits suitable for culture in the southern part of the cotton belt has been in progress for many years. The citrange, a cross of the hardy Chinese trifoliate orange, *Poncirus trifoliata* (L.) Raf. and the common sweet orange, *Citrus sinensis*, Osb. was the first of the hardy hybrids secured. The citrange was first reported on by the senior author and Dr. H. J. Webber[2] in the Yearbook of the Department of Agriculture for 1904, where the Rusk and Willits citranges, two that appear below as parents of new hybrids, were described and illustrated. It soon became evident that although the citrange is much like the trifoliate orange in its resistance to extreme cold when it is in a dormant condition, it is easily forced into a new growth in spring and sometimes is severely injured by untimely cold snaps. In consequence of this imperfect resistance of the citrange to spring and early autumn frosts, about 15 years ago the effort was first made to create new types of hybrids which would partake in some measure of another type of hardiness possessed very strikingly by the kumquat oranges. The kumquat is one of the hardiest of the evergreen citrous fruit trees; besides having a fair degree of resistance to cold it has an extremely high degree of winter dormancy. Both of the species of kumquat commonly grown in this country, the round and the oval kumquats, are able to pass unaffected through spells of hot weather which force other citrous into a tender and succulent growth that is liable to be injured by a slight frost.

The first successful hybrids wherein the kumquat was used with the idea of securing increased dormancy were made by the senior author in the spring of 1909. Four principal types of these kumquat hybrids were made: First, those between the trifoliate orange and the kumquat. These proved extraordinarily hard to make, and most of them were very weak, many of them dying while still small seedlings. None of these have proved as yet to be of any considerable value. Second, hybrids were made in considerable number between the more promising citranges, especially the Rusk and Willits, already widely cultivated in the cotton belt, and the kumquats. The hybrids resulting from these crosses are known as citrangequats, and are described in the present paper. Third the effort was made to hybridize the kumquat with the West India lime

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the lime being the tenderest citrous fruit grown in the United States, often being injured by cold even in central or south Florida. The hybrids resulting from this cross have been called limequats, and are also described here. Fourth, the attempt was made to hybridize the kumquat with the lemon, the lemon being almost as tender as the West India lime. None of these hybrids have been sufficiently well studied to warrant reporting on them as yet. So far as observed they have fruits very similar to the limequats described below except that the peel is much thicker.

THOMASVILLE CITRANGEQUAT

Among the citrous crosses made at Eustis, Fla., in June, 1909, by the senior author, was one in which pollen of the Willits citrange was used to fertilize flowers of the oval kumquat (*Fortunella margarita*) (Lour.) Swing.

Budded plants of the best of the hybrids resulting from this cross were sent to a number of cooperators in the southeastern States as soon as it was possible to effect propagation. The first plant to bear fruit was numbered C.P.B. 48010.[3] Fruits were received at Washington, D. C., in November, 1915, produced by a plant of this number that had been sent to Mr. P. J. Hjort at Thomasville, Ga., in March, 1913. As this hybrid was first fruited at Thomasville, where it was proved sufficiently desirable as a fruit for home use to induce Mr. Hjort to propagate it in his nursery, it is proposed to call it the Thomasville citrangequat.

The Willits citrange, the pollen parent, is itself a hybrid resulting from crossing the sweet orange (*Citrus sinensis*, Osb.) with the trifoliate orange (*Poncirus trifoliata* (L.) Raf.), the former supplying the pollen which was used on the pistils of the trifoliate orange.

While this citrange is of fairly good quality and makes an excellent ade, the oil of the peel is very bitter and ill-smelling, and a single drop is enough to ruin a pitcher of ade. It was hoped by hybridizing the Willits citrange with the oval kumquat to overcome this drawback of the citrange since the peel of the kumquat is so mild as to be edible, and at the same time the juice of the kumquat is pleasantly acid, so that a hybrid between it and the citrange would be expected to be suitable for making ade. These expectations were fully justified, as is shown below.

As was noted in the introductory paragraph of this paper, another quality that it was hoped to secure in this hybrid was an increased winter dormancy over that shown by the citrange. The kumquat possesses winter dormancy of a very high degree and is able to go through long spells of warm weather without starting new growth. The new growth of practically all citrous fruits is, of course, easily injured by even moderate frosts. Although the Willits, like other hybrids of the trifoliate orange, is extremely hardy when in a dormant condition, it is not able to stand long periods of warm weather in winter without starting new growth. The new citrange-kumquat hybrid, as was hoped, is superior to the Willits in all-around hardiness and shows a distinctly higher winter dormancy. Being the result of crossing a citrange with a kumquat, the new hybrid was called a citrangequat.

The citrangequat seedlings showed from the start, phenomenal vigor and in fact made the most rapid and continuous growth of all the nu-

[3] The abbreviation C.P.B. is used for the name of the Office of Crop Physiology and Breeding Investigations of the Bureau of Plant Industry, United States Department of Agriculture. All the numbers cited in this paper are of this series.

merous rapid-growing hybrids under propagation in the department greenhouses during the winter of 1909-10.

Plate 1 shows the vigorous growth manifested by a few of these citrangequats less than one year from the time of planting the seeds, the plants being, from left to right, 57½, 61, 49, and 52 inches high, while an oval kumquat of the same age (on the extreme right) was only 24½ inches high, only half the height of the smallest of the four citrangequats.

The Thomasville citrangequat is a hybrid of the oval Kumquat, *Fortunella margarita* (Lour.) Swing, and the Willits citrange, the last named being the pollen parent. The Thomasville and other citrangequats are what is known as trigeneric hybrids—that is, they were secured by hybridizing plants belonging to three distinct genera, Citrus, Poncirus, and Fortunella, as has already been noted. In this case the citrangequat is in effect a second-generation citrange—that is, a cross of the ordinary orange and the trifoliate orange, and a first generation cross of the oval kumquat. It is, therefore, one-half kumquat and approximately one-fourth each of common orange and trifoliate orange.

It is an interesting fact that, although the kumquat is a dwarf plant, nevertheless hybrids between it and the citrange have shown distinctly more vigor than similar hybrids between citranges and grapefruit, despite the fact that the grapefruit is a very much larger plant than the kumquat.[4] The vigor of the Thomasville and some of the other citrangequats is so great that their growth often exceeds the combined growth of the three parent species.

GENERAL FRUIT AND TREE CHARACTERISTICS OF THE THOMASVILLE CITRANGEQUAT

It was evident from an examination of the first fruits borne that a new type of citrous fruit had been created, having much of the pomological character of the lime, despite the absence of any lime blood. The fruits average much larger than the kumquat and are usually as large as the lime, which they resemble more closely than any other citrous fruit. Plate 2, A, B will give a good idea of the shape and size of the fruit and the manner of its attachment to the twigs. The pronounced "goose-neck" fruit spurs and clawlike calyx points are unique characters which make the fruit easy to identify.

The fruits are of attractive appearance, being a glossy dark green when immature, changing to a light yellow, and finally to an orange yellow when fully mature. They develop an abundant juice content when only half grown, a point of importance in their practical utilization. Though rather late in blooming, fruits develop very rapidly, in this respect resembling those of another hybrid, the Eustis limequat, also described in this paper. Although usually not mature until October or November, the fruits can be used for ade from July on through the summer and early autumn, when acid fruits are especially appreciated. When fully mature, they lose much of their acidity, and are sufficiently sweet to eat out of hand. By reason of its hardiness and pleasantly acid juice this new fruit makes available for home fruit gardens an ade fruit in regions much too cold for growing either lemons or limes. It has stood temperatures as low as 12° F. without injury and probably will

[4] This curious fact is perhaps explained by the fact that the kumquat is botanically very remote from the two parent species that enter into the citrange, whereas the grapefruit is closely related to one of the parents of the citrange—the common orange.

survive even lower temperatures. The evergreen nature of the tree also recommends it for home gardens or lawn planting. Judicious pruning or pinching back of long shoots will assist in giving the tree a fairly compact habit in place of its natural upright form.

The Thomasville citrangequat makes an excellent marmalade without the admixture of any other citrous fruits. The mildly pungent peel and the pleasantly flavored acid juice combine to give the marmalade an unusually good flavor, rivaling the best Dundee marmalade.

RESISTANCE TO DISEASE

The advent of Citrus-canker in the Gulf Coast States made it necessary to test any new Citrus varieties and hybrids with reference to their susceptibility to that disease before they could be recommended for culture.

As is well known, the kumquat is the most canker-resistant of all citrous fruits, its resistance amounting to practical immunity under field conditions. In this new hybrid, the Thomasville citrangequat, the kumquat parent has seemingly transmitted its remarkable canker resistance unimpaired, notwithstanding the fact that one parent of the citrangequat, the citrange, is decidedly susceptible to canker.

Several plants of the Thomasville citrangequat have been inoculated and continuously exposed to Citrus-canker for a period of four years without any canker having been developed except from needle punctures, from which canker failed to spread.[5]

It is highly important that only canker-resistant citrous fruits be propagated in the Gulf Coast States west of Florida, as a measure of insurance against a serious reinfection. From this viewpoint, the citrangequat becomes of great importance to this region, to meet the need for home-grown acid citrous fruits.

While somewhat affected by Citrus scab, the quality of the fruit is not greatly impaired thereby, and if planted away from scab-susceptible varieties it is probable that scab would not be at all serious on the citrangequat.

POSSIBLE USE OF CITRANGEQUATS AS A STOCK FOR SATSUMA ORANGES

The extraordinary vigor of growth, great hardiness, and extreme canker resistance shown by this hybrid suggested at once its possible value as a stock, especially for use in the Gulf Coast States west of Florida. In this region almost the only stock used for Satsuma and other Citrus varieties is the trifoliate orange, a very hardy, deciduous tree, closely related to the true species of Citrus. It is extremely thorny and is used extensively as a hedge plant, the fruits from these hedges providing an abundance of seeds to use in growing nursery stock. Unfortunately, the trifoliate orange is very susceptible to Citrus-canker. One necessary step in the eradication of canker is the complete elimination of these trifoliate orange hedges. The presence of large numbers of trifoliate seedlings in Citrus nurseries is also a menace in the event of a reappearance of Citrus-canker.

When considering the possible use of the citrangequat as a stock, the small number of seeds produced seemed an almost unsurmountable

[5] PELTIER, George L. SUSCEPTIBILITY AND RESISTANCE TO CITRUS-CANKER OF THE WILD RELATIVES, CITRUS FRUITS, AND HYBRIDS OF THE GENUS CITRUS. *In* Jour. Agr. Research, v. 14, no. 9, p. 350. 1918.

barrier, despite its promising features of canker-resistance and extreme vigor.[6] The recent discovery, however, that cuttings of the citrangequat may be rooted readily by the use of new methods, has been so encouraging that extended trials of this plant as a stock are now under way. By the use of rooted cuttings, practically a full year's time may be saved in growing stocks to budding size, an advantage certainly sufficient to offset any additional trouble and expense for greenhouse equipment. A combination of bottom heat, about 90° F, with humidity maintained at a high point, 80° to 90°, and with air temperature lower than that of the soil, 70° to 80°, is essential to the best results.[7] With good control of all these factors, small twig cuttings with leaves attached may be rooted within a few weeks—in less time, in fact, than it usually takes for seed to germinate.

Plate 3 shows two of these cuttings and the original seedling plants beside them, with the stubs visible where the cuttings were removed. These cuttings were photographed only 4½ months from the time they were placed in the rooting bed and are fine specimens of a large lot rooted in February, 1921. The seedlings were 18 months old. An interesting point in this connection is the fact that while the citrangequat roots so freely the three parent species utilized in creating the hybrid (the sweet orange, trifoliate orange, and kumquat) commonly fail to root under the same conditions.

By the use of rooted cuttings, certain advantages are secured well worth consideration. First, the time saved has already been mentioned; second, through vegetative propagation uniformity of vigor is secured which is highly desirable and cannot ordinarily be expected through the use of seedlings; and, third, it has often been noted in the propagation of cross-bred plants that there is a vigor of growth in the first generation seedlings not so apparent in succeeding generations of self-pollinated plants. This vigor can be utilized to best advantage by the rooting of cuttings from especially vigorous individuals.

Buds of Satsuma and other citrous fruits grow readily on citrangequat stocks, but tests under field conditions will be necessary before definite conclusions can be drawn. While such tests have been under way now for several years, more time will be necessary in order to secure trustworthy conclusions as to the effect of the stock on the quality, size, and season of fruit crops, as well as hardiness and other factors of practical importance.

Regardless of the possible utilization of the Thomasville citrangequat as a stock, its obvious value as a hardy, canker-resistant fruit tree for home use makes it desirable to give it a distinctive name and publish a detailed description.

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[6] The character of this hybrid as regards seed content seems to be dependent largely on the possibility of its receiving pollen from other seedy varieties of Citrus. In many places where grown by itself or along with seedless varieties (such as Satsuma, having little or no viable pollen) the fruit is practically seedless, while in other plantings where mixed with seedy varieties, seeds are usually found averaging six or eight to the fruit.

The Thomasville citrangequat, like many other hybrids between widely differing species of the Citrus group, reproduces itself unchanged from seed. This means that it is in reality a sterile hybrid and the embryo found in the seed is developed from the tissues of the mother plant and of course shows no effect of the pollen used in fertilizing the flower.

[7] The method used was developed in January and February, 1921, by Mr. Eugene May, jr., plant propagator of the Office of Crop Physiology and Breeding Investigations, and is an adaptation of the rooting methods devised by Mr. Edward Goucher, of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, United States Department of Agriculture, for the rooting of small twig cuttings of the Lychee. (GOUCHER, Edward. ROOTING LYCHEE CUTTINGS BY MEANS OF A HIGH TEMPERATURE AND HIGH HUMIDITY PROCESS. *In* GROFF, George Weidman. THE LYCHEE AND LUNGAN, Appendix 8, p. 157-159, New York, London, and Canton, China, 1921).

TECHNICAL DESCRIPTION OF THE THOMASVILLE CITRANGEQUAT

Fruit oval or nearly spherical, mature fruits having a long diameter of 1¾ to 2 inches, diameter in cross section 1½ to 1⅞ inches, calyx strongly persistent with usually 5 long, clawlike points; fruit spur long, swollen, and characteristically bent downward apparently by the weight of the fruit, color of mature fruit yellow to orange yellow,[8] rind thin (⅛ inch) somewhat roughened or "pebbly," oil cells small, depressed, segments, usually 7 or 8, thin-walled; core, little or none; seeds varying from none to 12, usually not more than 6, small and plump; pulp light greenish to amber color, juicy, pleasantly acid when immature, becoming sweetish when fully mature; tree evergreen, vigorous, of upright habit; thorns rather numerous on old wood but much reduced and inconspicuous on bearing twigs; leaves uni-, bi-, and trifoliate (on young plants chiefly trifoliate, on bearing wood chiefly unifoliate) long pointed oval or lanceolate, 2 to 3 inches in length, rather thick and stiff, slightly folded on the midrib, dark green, with finely incised margins; petioles, very narrow-winged; flowers small, pure white, 5-petaled, bloom appearing rather late though not as late as the kumquat, fruits ripening from October to December, but juicy and serviceable as ade fruits from July to October.

OTHER CITRANGEQUATS

During the spring and early summer of 1909, the senior author made many cross pollinations between citranges and kumquats. From these numerous crosses, 67 citrangequats were secured. These citrangequats are mostly hybrids of the oval kumquat with the citrange, the latter being the pollen parent. Five different citranges were used as pollen parents, but most of the citrangequats (49 in all) have Rusk citrange as the mother parent; the next largest group (11 in all) are hybrids between the Willits citrange and the oval kumquat. Three of the citrangequats are hybrids of the round kumquat pollinated by the Rusk citrange.

Not all of the citrangequats have been as yet carefully studied, but a number of them are of interest and several of the most promising are noted below.

A second citrangequat (C. P. B. No. 48011) was grown from the same crossed fruit that yielded the Thomasville. It differs from that variety in having large fruits more nearly spherical in shape and borne on upright fruit stalks instead of on curiously elongated "goose-neck" fruit stalks. This citrangequat, like the Thomasville, loses much of its acidity as the fruit matures and like it has a mild-flavored peel and usually few seeds. When fully ripe this citrangequat is sweeter than the Thomasville and can be eaten from the hand by those who like rather sour citrous fruits.

Another citrangequat resulting from the same cross of oval kumquat and Willits citrange is C. P. B. No. 48005. The pollen parent was the same as that of the Thomasville but the seed parent was a different tree growing in the same dooryard at Eustis, Fla. This citrangequat has attracted attention by fruiting freely in the garden of Mr. George H. Harris, a cooperator living at McRae, Telfair County, Ga., far to the north of the limit of ordinary citrous fruit culture.

It differs decidedly from the Thomasville in remaining intensely acid even when fully mature. The fruits are nearly spherical and are borne on erect fruit stalks. As this citrangequat has fruited successfully as far north as the latitude of Savannah, 32°, it seems advisable to give it a

[8] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE, pl. III, 15. Washington, D. C., 1912.

name, and it is accordingly proposed to call it the Telfair citrangequat (Pl. 2, C).[9]

Another citrangequat of the same parentage is C. P. B. No. 48007, which was conspicuous for its extreme vigor when young. (Pl. 1.) The fruit, like that of the Thomasville, acquires something of an orange flavor as it ripens. It seems to be an early ripening variety. The peel is mild flavored, and the seeds are few.

Still another citrangequat is C. P. B. No. 48050, a hybrid of the oval kumquat with the Rusk citrange. It attracted the attention of Mr. J. W. Benson, a cooperator living at Sinton, San Patricio County, Tex., near Corpus Christi, by its superior cold resistance and nearly seedless fruits. A tree that flowered in October, 1918, carried a crop through the winter unharmed. Samples received at Washington on April 23, 1919, were very like a lime in appearance and flavor, but showed a small, fleshy calyx, somewhat like that of the Rusk citrange parent. Fruits of this number, received November 1, 1920, from Glen St. Mary, Fla., were strikingly handsome, being bright orange or even flame scarlet in color. They were from 1½ to 1⅞ inches in diameter, nearly spherical, and had few or no seeds. These fruits were overripe, but the pulp was still acid, so this is doubtless an early maturing variety suitable only for ade.

As this citrangequat has attracted attention, not only because of its nearly seedless lime-like fruits produced in Texas, but also because of the extraordinarily brilliant color of the fully ripe fruits in Florida, it seems best to give it a name. It is accordingly called the Sinton citrangequat.[10]

EUSTIS LIMEQUAT

In 1909, the senior author originated a new type of citrous fruits by crossing the West Indian lime with the kumquat oranges. Those familiar with citrous fruits know that the lime is the tenderest of all the commonly grown species of this group. It frequently freezes to the ground even in southern Florida, so that its culture is chiefly confined to the fringe of keys along the coast. The kumquat, on the other hand, is one of the hardiest of the evergreen citrous fruit trees. This is without doubt not so much due to its direct powers of cold resistance as to its remarkable dormancy. The kumquat, as noted above, is able to pass unaffected through long spells of hot weather that force other Citrus trees into a tender and succulent growth that is liable to be injured by even a light

[9] TECHNICAL DESCRIPTION OF THE TELFAIR CITRANGEQUAT: Fruit spherical (sometimes slightly flattened or elongated) mature fruits having a diameter of 1¼ inches to 1½ inches; calyx persistent with 5 long points; color of mature fruit light orange yellow to deep chrome (RIDGWAY, Robert, OP. CIT., pl. III, 17, b, d); rind thin, somewhat roughened; oil cells prominent, small, numerous; core little or none; segments 4 to 6 (usually 5), seeds few, variable in size, usually undeveloped, pulp pale yellow; Pinard yellow, (RIDGWAY, Robert, OP. CIT., pl. IV, 21, d.), sharply acid, and retaining this quality when mature; rind slightly bitter; tree evergreen, vigorous, of upright habit; leaves uni-, bi-, and trifoliate, generally obovate, 1½ to 2½ inches in length, finely serrated; plant fairly thorny, thorns smaller in size on the fruiting twigs; bloom often occurring over a long period, giving a semieverbearing fruiting habit.

[10] TECHNICAL DESCRIPTION OF THE SINTON CITRANGEQUAT: Fruits varying from slightly flattened to slightly elongated; growing in clusters; diameter varying from 1½ inches to 1⅞ inches; calyx small, persistent, slightly protruding, sunken pistil surrounded by a very small raised circle; color variable, ranging from a light orange-yellow (RIDGWAY, Robert, OP. CIT., pl. III, 17, d), to a light flame scarlet (RIDGWAY, Robert, OP. CIT., pl. II, 9); rind very thin, smooth, highly colored, 1/16 inch in thickness and much like kumquat, showing almost none of the flavor of the trifoliate orange; oil cells few, variable in number; segments 7 to 8; no seed in 100 of fruits, occasionally one or a few; pulp pale, varying from orange buff (RIDGWAY, Robert, OP. CIT., pl. III, 15, d), to a light cadmium (RIDGWAY, Robert, OP. CIT., pl. IV, 19); flavor sharply acid, decidedly lime like with only faint suggestion of citrange flavor given by the oil from the rind, no citrange flavor in the juice; makes a good ade; core very small and segment walls very thin; tree much more hardy than the lime; bearing twigs spineless; leaves on bearing branches unifoliate, rather thin, 2 to 3 inches in length by 1¼ inches in width; petiole ½ inch long, slightly winged; midrib heavy, prominent on upper surface; leaves tapering slightly toward the apex.

frost. Some measure of the dormancy of the kumquats is indicated by the fact that they flower from two to three months later than other citrous trees.

This series of crosses between the lime and the kumquats was made at Eustis, Fla., in June, 1909, in the Citrus grove of Mr. F. W. Savage. These crosses resulted in a number of hybrids varying in character, but all having fruits much like the lime in quality. Attention has been attracted to one of these hybrids (C. P. B. No. 48798) that fruited during the summer of 1918 in the greenhouse of the Department of Agriculture at Washington, D. C. It resulted from fertilizing the flowers of the common or West Indian lime with pollen of the round kumquat.

The Eustis limequat fruit is of striking appearance, strongly resembling a West India lime in color, size, and texture. (Pl. 4.) The color is a light yellow, resembling the color of grapefruit. When cut, the fruit shows its lime-like character. It is very juicy, thin-skinned, has few seeds, and the flavor can hardly be distinguished even by an expert from the true lime. Moreover, the rind is edible, like that of the kumquat, so that the whole fruit may be utilized. It is particularly promising as a fruit for crystallizing. The spines on the bearing wood are very inconspicuous, a decided point in favor of this hybrid in contrast with the viciously spiny character of the common lime. Some of the other limequats exhibit rather long spines, even on the small twigs. Observations covering several seasons indicate that this limequat possesses much of the immunity to disease and insect pests enjoyed by the kumquat. The kumquat is the most resistant to Citrus-canker of all citrous varieties, a resistance amounting to practical immunity, an advantage the limequat has in some degree.

Specimens submitted to Citrus experts and growers have in every case elicited favorable opinions, it being held that the limequat's place in citrous fruit culture is not dependent entirely upon unusual hardiness. Even should it prove no more hardy than the sweet orange, it would still be of great value throughout the orange-growing regions, as the lime is so extremely tender that its culture is now almost entirely confined to the protected keys along the Florida coast. This hybrid, as well as several sister hybrids of this limequat, have been fruited at Glen St. Mary, Fla., in the northern tier of Florida counties, but were killed in the successive freezes of 1917 and 1918. With a little protection while young, and during unusually severe weather, it is probable that this hardy form of lime could be grown in the warmer parts of the Gulf Coast, supplying a real need in this region.

In Florida, California lemons are not obtainable owing to the quarantine restrictions necessary to prevent the introduction of "brown rot." Sicilian lemons are often expensive and are only obtainable in the larger towns, so that it often happens that a good acid citrous fruit for ade making is a decided scarcity even in Citrus-growing territory.

Budding experiments on different stocks are in progress and the present indications are that the limequat will thrive on most of the stocks commonly used except sour orange. Unlike the Thomasville citrangequat the Eustis limequat does not come true from seed. Nursery propagation is chiefly confined to the rough lemon and trifoliate orange stocks. Like the lime, if planted low and mounded with soil the limequat bud will strike root, thus simplifying the stock question. Its fruiting season is also of importance. To be of the greatest service in the home fruit garden it should bear the bulk of its crop in the warm summer months.

The indications are from the few trees that are in bearing in Florida that it will prove more or less everbearing, following the lime parent in this regard. No doubt its fruiting season can be influenced by methods of fertilization and pruning. Selection of budwood from early or summer-bearing trees will also be desirable.

The limequat promises to become a valuable addition to our list of citrous fruits. Since the original cross pollination resulting in this new fruit was made at Eustis, Fla., a well-known Citrus-producing center, it is proposed to name this hybrid the "Eustis limequat."

TECHNICAL DESCRIPTION OF EUSTIS LIMEQUAT

Fruit oval (occasionally nearly spherical) slightly asymmetrical, mature fruits having a long diameter averaging 1⅝ to 1⅞ inches, diameter in cross section, 1⅛ to 1½ inches; calyx persistent; color light yellow ("picric yellow")[11]; rind thin, $\frac{1}{16}$ inch, very smooth and glossy, translucent; oil cells spherical, rather prominent, having no bitter flavor; segments 6 to 9; seeds 5 to 12, usually averaging 1 to the segment, small to medium size, ¼ to ⅜ inch in length; pulp of light greenish color, closely resembling lime, tender and juicy, flavor sharply acid (like the lime the fruit can be used when full sized but not fully colored); tree evergreen, vigorous, having a tendency to produce long shoots which bend downward with the weight of the fruit; resembles the lime in producing a succession of crops, though not so markedly everbearing; spines on bearing twigs very small, not injuring the fruit; flowers 5-petaled, pure white (not streaked with pink as those of the lime and of most other limequats); leaves unifoliate, rather thick, dark green above, lighter below, tapering at both base and apex, 2 to 3 inches long, slightly folded along the midrib, having short, narrow petioles, closely resembling leaves of the round kumquat except for larger size.

OTHER LIMEQUATS

In addition to the Eustis limequat, some 28 other hybrids were made by the senior author in 1909 between the West India lime and the kumquat. Twenty-four of these, like the Eustis, are hybrids of the West India lime and the round kumquat; four are hybrids of the West India lime and the oval kumquat. Only about one-third of these have been studied in detail, but several of them are being grown in Florida and other Southern States. Perhaps the most promising of these is a limequat (C. P. B. 48786) of the same parentage as the Eustis; that is another cross between the same two parent trees. This is the largest limequat yet produced, sometimes equaling a small lemon in size, usually being about 1¾ inches in diameter and 2¼ inches long. The fruits, bright yellow when ripe, have a very pale pulp of a pleasant acid flavor, similar to the Mexican lime, the skin is very thin but tough and of a mild flavor. There are from two to nine seeds in a fruit.

This limequat has done particularly well in the garden of Hon. H. J. Drane at Lakeland, Fla., and as it is likely to be propagated more or less it seems desirable to give it a name. It is accordingly called the Lakeland limequat (Pl. 5, A).[12]

Another limequat that has attracted much favorable notice is C. P. B. No. 48792. This is a hybrid of the Mexican lime with the oval kumquat,

[11] RIDGWAY, Robert, OP. CIT., pl. IV, d 23.

[12] TECHNICAL DESCRIPTION OF LAKELAND LIMEQUAT: Fruits very smooth, attractive in appearance, oval, average 2¼ inches long 1⅝ inches in diameter; calyx not persistent; color Strontian yellow (RIDGWAY; Robert, OP. CIT., pl. XVI, 23); rind very thin, smooth, sweet and edible like that of kumquat; oil cells large and prominent, transparent, showing through the rind, segments 5 to 8, seeds 2 to 9, large and oval, usually about 6, pulp pale, massicot yellow (RIDGWAY, Robert, OP. CIT., pl. XVI, f, 21), resembling lime, very juicy, sharply acid, that of lime; good before fruit is fully colored; leaves 2½ to 3 inches in length; evergreen, unifoliate, lanceolate acuminate, petiole short, very narrowly winged; tree vigorous, bearing wood nearly spineless.

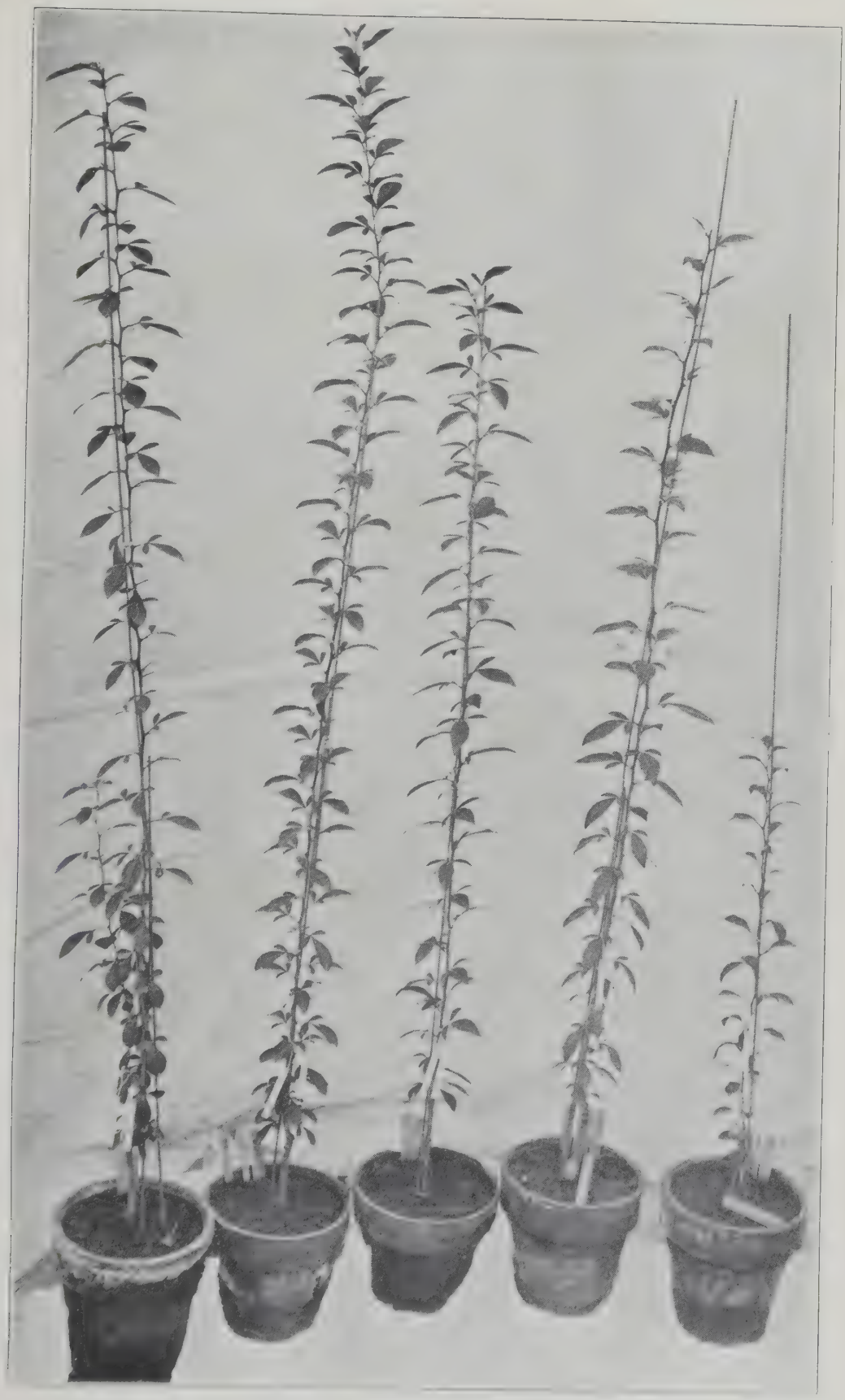
whereas the Eustis limequat and the Lakeland limequat are hybrids of the Mexican lime with the round kumquat. As this limequat has already been propagated to some extent in Florida and is certain to become more or less widespread as a dooryard fruit, it seems best to give it a name. It is accordingly called the Tavares limequat,[13] Tavares being the town adjoining Eustis, between which two cities lies Mr. Frank W. Savage's orange grove, where this and all the other limequats were produced.

The fruits are very similar to those of the Eustis limequat in appearance, and have much the same flavor. (Pl. 5, B.) It is often difficult to distinguish the fruits of these two varieties, the Tavares and the Eustis. On the whole the Eustis seems to be rather better in quality than the Tavares. The two can easily be distinguished by the fact that the flower buds of the Eustis are pure white while those of the Tavares have a pinkish coloration, like those of the West Indian lime.

[13] TECHNICAL DESCRIPTION OF TAVARES LIMEQUAT: Fruits obovate or oval, occasionally nearly spherical; 1¾ to 1 5/16 inches long, 1¼ to 1 7/16 inches in diameter; light cadmium yellow (RIDGWAY, Robert, OP. CIT., Pl. IV, 19'); rind, very thin, smooth, tender, edible, averaging 1/16 inch in thickness, mild flavored, stronger and tougher than that of kumquat and other limequats; segments 7 to 8; segment walls thin; seeds large, 6 to 11, usually 8 or 9, oval, often pointed at one end; pulp mustard yellow (RIDGWAY, Robert, OP. CIT., Pl. XVI, b 19'), resembling lime; juicy, sharply acid; leaves evergreen unifoliate, lanceolate; petioles very narrowly winged; tree vigorous with short spines on bearing wood; flower buds pink

PLATE 1

Four citrangequats and one oval kumquat, nearly 1 year old; nurse-grafted when 2 months old, on pummelo stock. From left to right C. P. B. No. 48005 (Telfair); No. 48007; No. 48010 (Thomasville); No. 48036; No. 48049 (oval kumquat). About $\frac{1}{10}$ natural size.



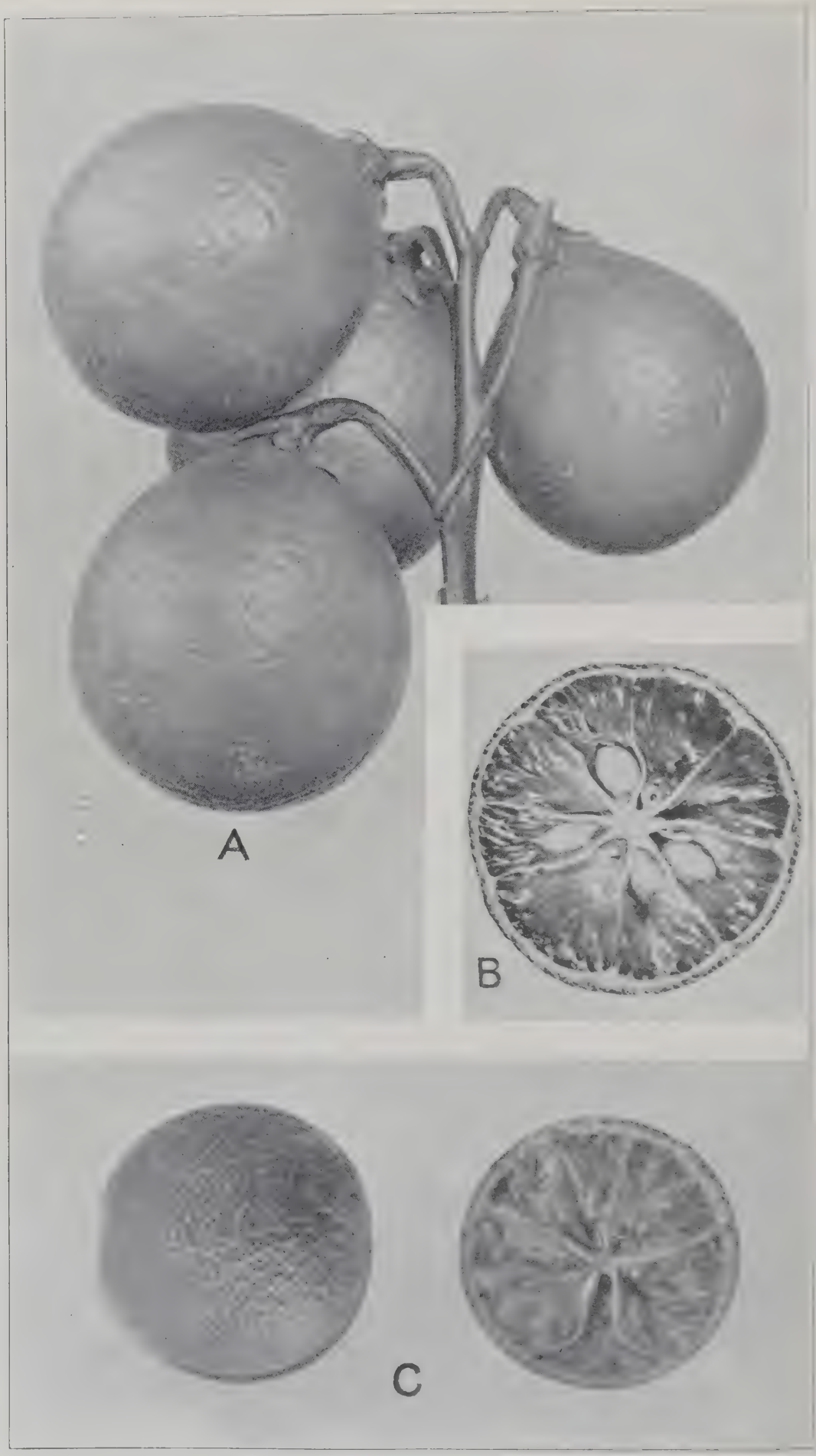

A
B
C

PLATE 2

A.—Thomasville citrangequat (No. 48010). Natural size.

B.—Cross section of Thomasville citrangequat sent in by Mr. A. M. Troyer to the Bureau of Plant Industry, from Fairhope, Ala., February 1, 1922.

C.—Telfair citrangequat (No. 48005) from McRae, Telfair Co., Ga., September 28, 1921. Natural size.

PLATE 3

Two Thomasville citrangequat cuttings (at left and right) each 4½ months old. In the middle two 18-months-old seedlings, showing the stubs from which the cuttings were taken. About ⅛ natural size.

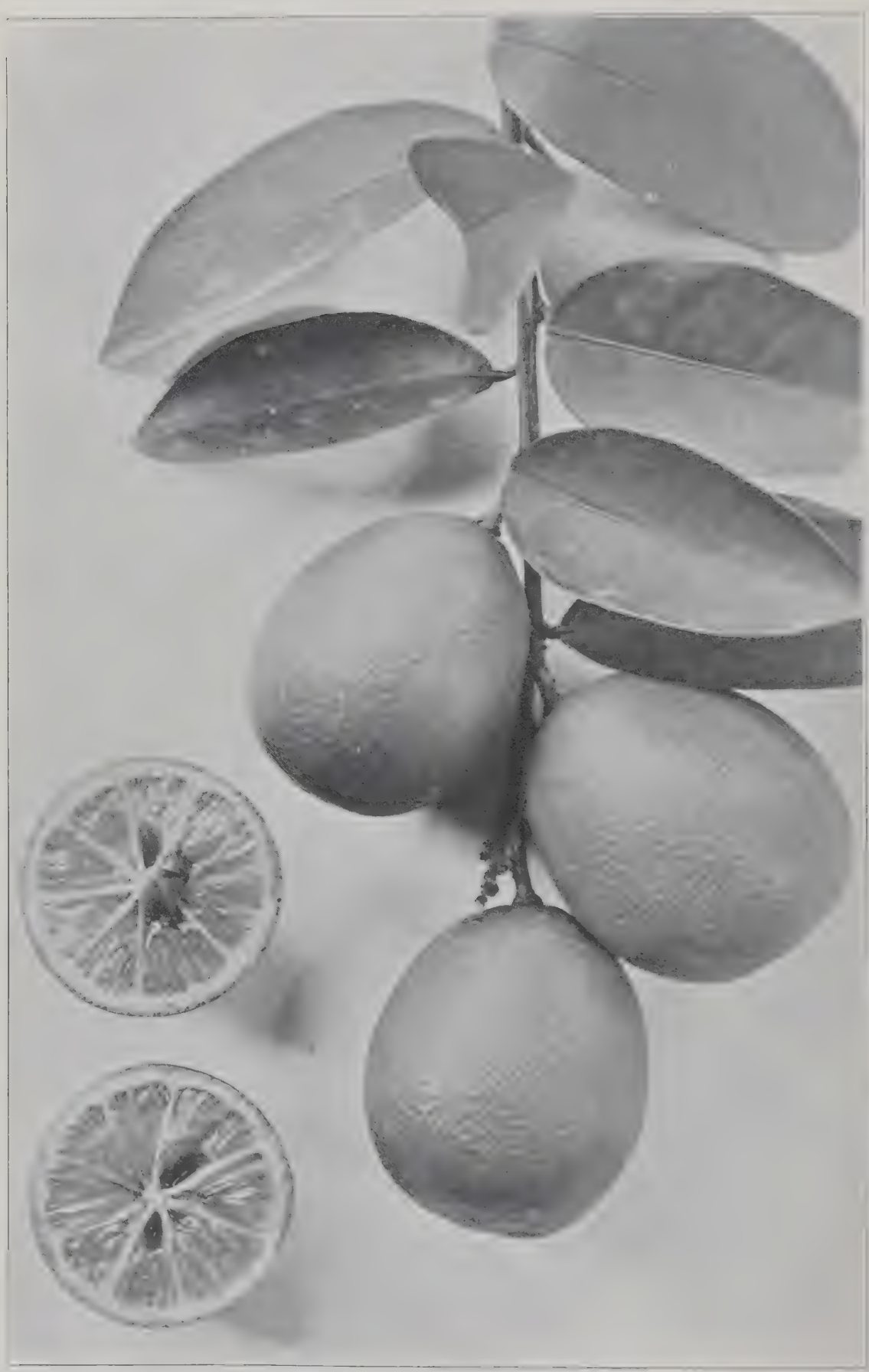
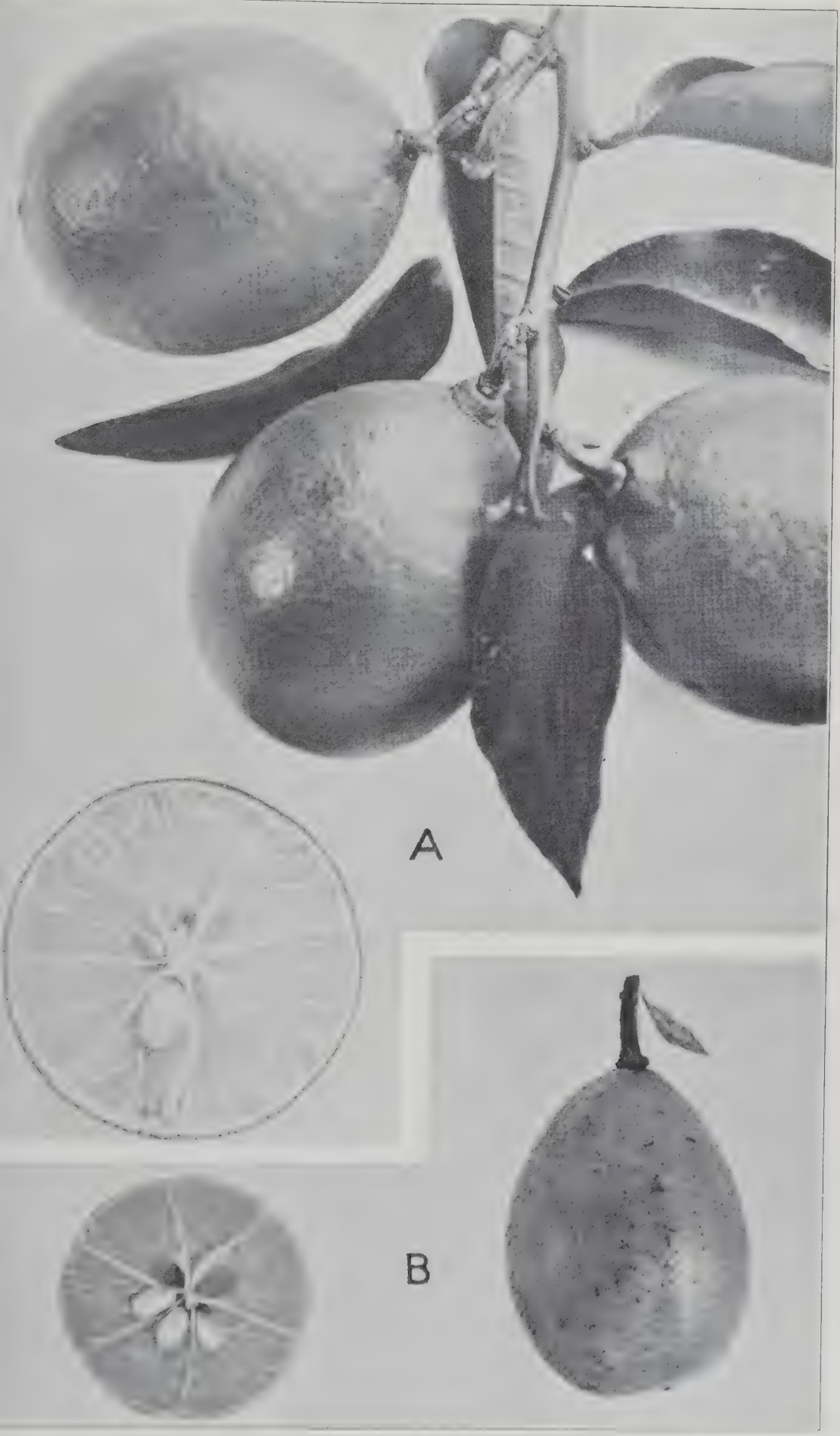


PLATE 4

Evstis limequat (No 48798), grown in the greenhouse at Washington, D. C. Natural size.

PLATE 5

A.—Lakeland limequat from Lakeland, Fla., November 6, 1919.
B.—Tavares limequat from Glen St. Mary, Fla., December 23, 1921.


A
B

PRODUCTION AND DISPERSAL OF CONIDIA IN THE PHILIPPINE SCLEROSPORAS OF MAIZE [1]

Pathologist in Charge of Downy Mildew Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

By WILLIAM H. WESTON, JR.[2]

INTRODUCTION

The members of the genus Sclerospora have come to be recognized as among the most destructive of the downy mildews (Peronosporaceae). They long have been responsible for considerable injury to such cereals as millet and wheat in Europe and have recently occasioned alarming losses of maize and related crops in the Orient.

The genus is known to produce two types of spores; conidia which are not at all hardy but serve to spread the disease rapidly under favorable conditions, and resistant oospores which enable the fungus to persist through such unfavorable circumstances as drought, extremes of temperature, or freezing. Unfortunately, however, comparatively little has been known of the methods of spore production and dispersal in the case of these fungi, and it can even be said, as Butler (*3*),[3] who has contributed largely to our knowledge of the oriental forms, remarks:

> The life history of the cereal downy mildews, which all belong to the present genus, is the most obscure among the Peronosporaceae.

By the early European workers who described the type species (*Sclerospora graminicola* (Sacc.) Schroet.) on which the genus Sclerospora was founded, most of the emphasis was laid on the oospores, and very little attention was paid to the conidial stage, the meager descriptions and illustrations of this phase obviously depicting herbarium material unfavorable for study (*11*, p. 437–439, fig. 7). Recent investigation of the several downy mildews of the Orient, however, has of necessity dealt chiefly with the conidial stage, as in almost all of these cases the oospores apparently play little, if any, part in the life history. As a result, our appreciation of the importance of the conidial phase of Sclerospora has been increased, and our knowledge of the characteristics of the conidiophores and conidia has been augmented by further information.

Unfortunately, however, the available information concerning the conidial stage of the Sclerosporas is still all too scanty. For instance, no data have been furnished on the process of conidiophore and conidium production, its duration, its amount, and its relation to environmental conditions. Yet such information is obviously of primary importance, as spore production and dispersal are intimately related to the distribution and severity of the disease; and in a complete knowledge of all phases of these processes lies the means of intelligently effecting control.

During the past two years the writer studied the destructive downy mildews of maize which are common in the Philippines and found that

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[3] Reference is made by number (italic) to "Literature cited," p. 277–278.

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two closely related but specifically distinct conidial Sclerosporas were causal there. One of these, *Sclerospora spontanea* Weston, occurs on maize and the wild grass *Saccharum spontaneum L.* in the Visayan Islands and is particularly distinguished by its long, slender conidia; while the other, *Sclerospora philippinensis* Weston, occurs on maize in the Island of Luzon and has relatively short conidia. Papers describing the characteristics and discussing the destructiveness, distribution, and relationship of these species already have been published (*24*, *25*).

In the course of this work the process of conidium production and dispersal has been worked out; and as this phase of the life history of Sclerospora has never before been investigated in detail and shows several points which are of considerable importance in their relation to control, and of some interest from their mycological aspect, these are presented in the following paper.

PRODUCTION OF CONIDIA

As has been reported previously by the writer (*24*, *25*), the conidial stage is the important one in the life history of the Philippine maize mildews, as it is by means of this stage exclusively that the widespread and serious destruction of maize is accomplished in the Philippine Islands, the oospores being as yet unknown on this host. The most vital phase of the conidial stage is the complex and hitherto obscure process of conidiophore and conidium production. Detailed consideration of this matter necessitates discussion of the following points: the demarcation of the conidiophore-bearing areas through preliminary paling of the infected tissue; the influence of environmental conditions, especially moisture, in inducing conidiophore production on these areas; and the emergence and development of the conidiophores thus induced. In addition, the nocturnal cycle of conidiophore development under various conditions, the long duration of this production in relation to the life of the host, and the magnitude of the numbers of conidia produced will be considered. Because the two causal species of Sclerospora agree essentially in all the main features of conidium production, the following account of the process except as noted applies equally to both.

PRELIMINARY PALING OF THE CONIDIOPHORE-BEARING AREAS

When a maize plant becomes infected with Sclerospora, it first develops on the normally green leaves markings of a more or less conspicuous pale yellowish white color (*Pl.* 1 *B*), and later it shows on the areas thus marked a down or felt of innumerable conidiophores and conidia (*Pl.* 1, *A*). These etiolated markings are always present in infected plants but vary greatly in extent, in shape, and in color according to the effect of such factors as the individual and varietal character of the host, the age at which it became infected, and the kind of climate and soil which have influenced it subsequently. As a result, it is not possible to cover the range of variation by any general description of the markings. To be sure, they may be grouped roughly into such categories as the types 1, 2, and 3 recognized by Palm (*16*) in the Javan maize mildew; but it should be remembered that these types are not differentiated with absolute distinctness, but intergrade.

Certain types of markings are not associated with conidiophore production and hence need not be considered here. Palm's third type, with slender golden striping decreasingly extensive on successive leaves, and the markings of similar characters found by the writer (*25*) to result from

infection through suckers, as well as the inconspicuously marbled markings described later in this paper as the result of preventing the access of dew or other moisture to infected leaves, are of this kind.

Certain markings, however, are always associated with conidiophore production. The most common of these have been described and illustrated by the writer (*24*) for the maize downy mildew of the Philippines, by Butler (*3*) for that of India, and by Palm (*16*) and Rutgers (*20*) for that of Java. The photographs, diagrams, and colored plates in these papers show the general characteristics and the range of variation in color and pattern of these markings. Consequently, no further description is necessary at this point.

It should be noted, however, that as a rule the markings associated with conidiophore production are progressively more extensive on successive leaves. This is especially noticeable in those markings which, because of their extremely pale color and large areas, are particularly conspicuous; and which, because they are associated with excessively abundant conidiophore production, are of especial concern to us here. In general, a leaf showing such marking has at the base a more or less extensive solidly discolored area from which irregular, jagged, tongued protrusions extend toward the tip. On the lowest leaf attacked (which may be any from the second or third up to the eighth or ninth) this discolored area is confined to a small portion of the base of the blade, while on each successive leaf the area increases in extent and the irregular protrusions run farther and farther progressively toward the tip, until on the last leaves the yellowish white discoloration occupies the whole blade (fig. 1).

The development of this pale color throughout these leaf markings of the diseased plant is a gradual one. When infected but as yet normal plants are kept under constant observation during the appearance of the disease symptoms, it is noticed that in the normal dark green of the leaves that are already unfolded, areas of the characteristic shapes that have been described, pale gradually through a yellowish green, then yellow, to a yellowish white color. The process is usually a gradual one, requiring several (6 to 24) hours from the beginning of the first difference in shade until the assumption of the final persistent whitish color. Since this loss of color is due to changes in the chlorophyl-bearing cells of the leaf brought about by the invading hyphae of the parasite, it is remarkable that so decided an alteration can be effected in what is relatively so short a time. The whitening of these areas may take place apparently at any hour, but in most cases observed it has been seen to occur during the night or in the early morning.

It should be emphasized particularly that the extent of these discolored areas is established at the beginning, and no increase in size takes place as time goes on. If, as soon as the discolored areas of an infected plant have paled sufficiently to be distinguished, they are outlined lightly with waterproof ink, it can easily be seen that when once defined they never grow out toward the tip of the leaf, but their outlines remain unchanged in extent. Moreover, if in a downy-mildewed plant one dissects out the young leaves, on the tips of which the markings can be seen as they unroll from the bud, the discolored areas on the inclosed part of the leaf will be found already faintly defined. These faintly distinguishable areas may, to be sure, expand somewhat as the unrolling leaf expands to its normal dimensions, but they show no progressive growth once the marked areas are established.

RELATIONSHIP OF CONIDIUM PRODUCTION TO ENVIRONMENTAL CONDITIONS

After the infected corn plant has developed the peculiar yellowish white markings which are characteristic of the disease, the production of conidiophores and conidia on these areas begins. Just how soon this can occur has not been determined; but in most of the cases observed, in

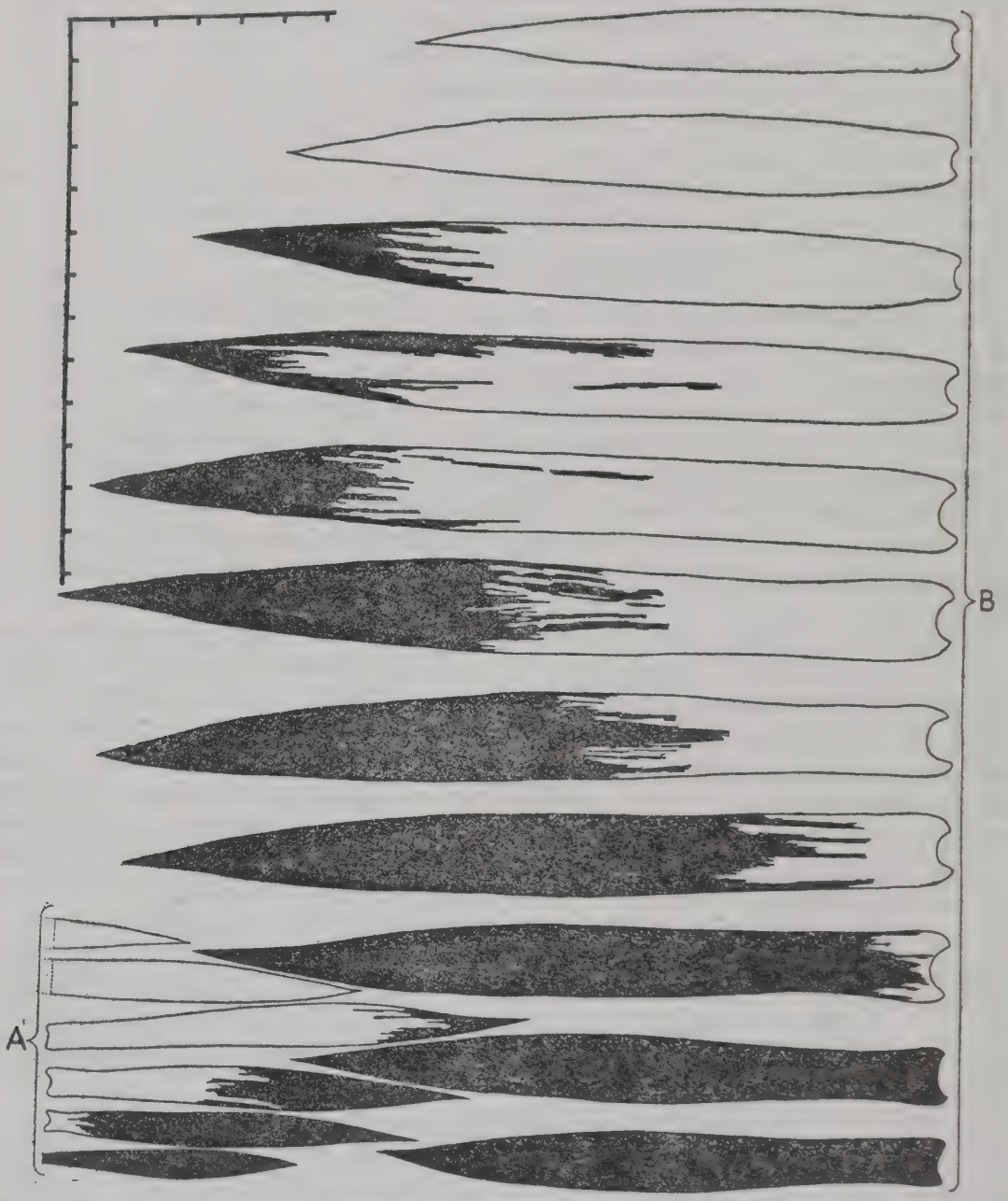

FIG. 1.—Diagrams (from tracings) showing the characteristic configuration and extent of the etiolated conidiophore-bearing areas on successive leaves of conspicuously diseased maize plants (A, plant 1; B, plant 10, Table II). The etiolated area (white) is increasingly greater and the unaffected area (black) increasingly smaller on successively developed leaves. The proportion of the total surface which produces conidia is relatively large. Scale in inches.

which the paling of the symptomatic markings took place at night or in the early morning, conidium production from these diseased areas began the next night if environmental conditions were immediately favorable. Under less favorable circumstances, however, several nights sometimes elapsed before conidia were produced.

The production of conidia from the diseased plant takes place almost without exception at night. Of the conditions which obtain during the night in the Philippines, the most essential to conidiophore production is apparently the presence of a layer of moisture on the surface of the infected plants. In this regard dew plays the foremost part under customary field conditions, while gentle rains are of somewhat less importance. The film of moisture must not be temporary but must persist for at least four or five hours to permit the conidiophore buds to protrude from the stomata, develop into conidiophores and mature conidia. The maximum production of conidia is attained when the whole plant surface is drenched and dripping with a heavy deposition of dew, as happens when still, clear, cool nights follow hot, rainy days. A gentle, drizzling rain persisting through the night and keeping the surface of the corn plant covered with a layer of moisture also results in conidium formation, though to a less extent. During violent, nocturnal storms, however, when the plants are beaten with driving torrents of rain, comparatively little conidium production occurs, and then only on the more protected parts of the plant where the delicate conidiophores can develop uninjured. No conidium formation takes place on nights when there is no deposition of dew on the infected plants, or when the temporary surface layer of moisture, whether of dew or rain, is dried in a short time by inopportune winds.

That the nocturnal production of conidia by the downy mildew depends on the presence of a layer of moisture over the host leaves was found to hold true not only under natural conditions in the field but also under the controlled conditions of various experiments. A number of maize seedlings, after being inoculated with the downy mildew, were divided into two lots, one of which was exposed to the usual field conditions, while the other was kept indoors protected from dew, rain, or other surface moisture. After the normal incubation period both lots developed the discolored markings characteristic of the disease; but although on successive moist nights the exposed plants produced abundant conidiophores, the plants indoors, on the contrary, consistently failed to produce any conidiophores whatever. After this had gone on for 10 days a few plants from the exposed lot were brought indoors, and a like number of the indoor lot were placed in the field. On the first favorably moist night thereafter conidiophore production took place on the latter newly exposed plants, although previously, while indoors, this had never occurred. On the contrary, the plants which had been supporting abundant conidiophore production in the field now, when brought indoors, ceased to bear any conidiophores whatever. Those other plants of the original lots which had been left indoors or had remained in the field continued to behave as heretofore. By varying the procedure in a series of experiments such as this, the dependence of conidiophore production on nocturnal moisture on the host was clearly demonstrated. When once this dependence was realized, it was comparatively simple to manipulate conditions so that the formation of conidiophores was induced or stopped at will.

It should be noted, however, that conidiophore production on an infected plant can not thus be induced month after month indefinitely, but if continued at intervals in experiments will gradually cease, being limited in duration as it is in plants growing undisturbed in the field. For example, if under field conditions the production of conidia

on a set of plants extends over two months, then this time will be the limit for inducing conidium formation on a similar set of plants which after inoculation have been kept indoors except when exposed to dew for occasional tests. It should be noted also that the markings of the disease on inoculated plants indoors are relatively inconspicuous when they appear and gradually become less and less marked until eventually they are so greenish in color and so broken up and interrupted in pattern that they are only recognized with difficulty.

In general, conidium production by the downy mildew takes place from the surface of the host chiefly on the discolored areas characteristic of the disease (Pl. 4, B, C, D). It may also occur, although less abundantly, on areas of the host that are apparently normal. Microscopic examination shows that the discolored areas harbor the most abundant mycelium in their tissues; but even areas that are seemingly healthy, and in fact any part of the diseased plant except the roots, may be invaded to some extent by the Sclerospora hyphae. As a result, in the case of plants which have become infected after they have attained sufficient growth so that they can struggle along to maturity in spite of the parasite, abundant conidiophore production may occur not only from the leaves but also from the leaf-sheaths (Pl. 4, E), from the husks Pl. 5, B), from the abnormal bract-like structures which are produced by diseased tassels and ears (Pl. 5, A), from the upper internodes of the stem, from normal parts of the tassel, and from the green, exposed tips of infected ears.

Conidiophore production may take place on both surfaces of the leaf. Whether the conidiophores emerge from the upper or lower surface of a leaf depends first, on the presence of stomata through which the conidiophores may develop, and, second, on the presence of a sufficient layer of moisture to induce this development. In maize the distribution of the stomata is rather uniform, the number in the lower surface of the leaf exceeding only slightly those in the upper. As a result, if other conditions are equal, the abundance of conidiophore production may be somewhat greater from the lower surface, but as a rule it appears to be about the same on both.

The presence of a layer of moisture on the leaf surfaces is determined by climatic conditions. During a gentle, drizzling, nocturnal rain there is deposited on the exposed upper surface of the leaf a sufficient layer of moisture to induce conidium formation there, while the protected lower surface is for the most part dry and bears no conidiophores. Dew, however, is usually deposited rather uniformly over the infected plant, on both the upper and lower surfaces of the leaves, and hence induces conidiophore production which either is approximately equal on both surfaces or else is somewhat more abundant on the lower, because of the more numerous stomata there.

This relatively even distribution of the conidiophores induced by dew is the type most frequently encountered on maize in the Philippines, and the reports of Butler (*3*), Rutgers (*20*), and Palm (*16*) that conidiophores occur on both surfaces but are slightly more abundant on the lower would indicate a similar condition in the case of the other oriental maize mildews. On sugar cane, however, at least on the only conidiophore-producing plant which the writer found in the Philippines, the conidiophores are almost wholly confined to the under surface of the leaves, and this is true also of the wild grass *Saccharum spontaneum*.

That this is not due to any character inherent in the fungus is shown by the fact that both species of conidial Philippine Sclerosporas behave in this way when growing on *S. spontaneum* and yet develop conidiophores on both sides of the leaves when growing on maize. On the contrary, the restriction to the lower surface of the leaves of sugar cane and the related wild Saccharum is attributable to the fact that in this genus by far the greater proportion of stomata are on the under surface. Dickhoff (*6*) and others have found this condition in sugar cane, and the writer has corroborated it in the wild Saccharum. It is of interest to note also that Miyake (*15*) reports that the Formosan downy mildew forms conidiophores only on the under surface of the leaf in sugar cane but on both surfaces in maize and teosinte.

When the production of conidiophores and conidia by the Sclerospora mycelium in a leaf first begins, it is not usually abundant. It starts on the first night with the protrusion of a few scattered conidiophores from the surface of a badly affected area usually along or adjacent to the midrib. During succeeding nights more and more conidiophores appear until finally vast numbers are produced nightly. As a rule, conidiophores appear first on the lowest leaves and leaf sheaths, which are markedly affected, and later develop progressively upward on the younger leaves of the plant.

After discovering that conidiophores were produced by the downy mildew only at night, the writer naturally endeavored to determine what factors operative during the night served to induce the mycelium of the fungus to send out conidiophores through the stomata of the host leaves. The conditions of the environment which prevail at night are darkness, lowered temperature, and the deposition of a layer of moisture on the surface of the host plant; the plant's stomata are nearly or quite closed, and metabolism is altered after the cessation of the photosynthesis which went on during the day. Hence, changes follow in the chemical content of the host cells and even of the gases in the substomatal chambers.

An attempt was made to determine by experiment which of these factors, or combination of interacting factors, was involved. It was found that darkness was not operative alone, nor in conjunction with the closed stomata and the metabolic changes following cessation of photosynthesis. No conidia were produced during the day time on infected plants kept in dark chambers, either at normal temperatures or cooled by ice; nor at night on plants which were cooled naturally and which had been undergoing normal photosynthesis in the sun during the day. Hence, it seems logical to conclude that neither darkness alone, nor darkness and lowered temperature combined, nor darkness and lowered temperature following normal exposure to the sun were the essential factors involved. Because in the first two instances the stomata were closed in plants in the dark chambers, and because in the third case the stomata were closed and the condition of the cells was the normal one after photosynthesis had ceased, it seems doubtful if these factors also are of any particular influence in conidiophore production.

The remaining factor, however, the presence of a layer of moisture deposited on the surface of the infected plant, does seem to be directly connected with conidiophore production. The writer found by a number of experiments, some of which have already been described earlier in this paper, that apparently it is the dew or other moisture on the surface of

the plants which induces the nocturnal conidiophore production. Just how this layer of moisture operates in this connection is unknown. Possibly the layer of moisture is effective by sealing the stomata and influencing the gas or moisture content within the substomatal chambers, so that this in turn affects the branches of the mycelium located there. It seems more probable to the writer, however, that this layer of moisture covering the stomatal pores furnishes the emerging conidiophores of the Sclerospora the necessary moisture for their development and that this development can not take place in the air alone. If this is the case, it arouses the interesting conjecture that this relationship may be of phylogenetic significance, an indication of steps by which the terrestrial Peronosporaceæ have been evolved from aquatic saprolegniaceous ancestors.

However, attempts to induce conidiophore production during the day by supplying a layer of surface moisture to the infected plants were entirely unsuccessful. Plants which had been actively producing conidiophores night after night were put in an ice chest and kept at a lower temperature for some hours; but although moisture was deposited on the inner walls of the container, the leaves, probably because their respiration prevented them from reaching the dewpoint, remained free from dew. The surface of the infected plant was, therefore, kept covered with a thin layer of moisture by repeated spraying. No conidiophores, however, were formed, although a darkened ice chest and also a light double-walled glass chamber maintained at the same temperature were used in the experiments. Moreover, heavily infected leaves of such plants when fastened so that they were submerged in jars of pure water produced no conidiophores either during the day or at night. These experiments are admittedly crude, and it is probable that the processes resulting in conidiophore production in the surface layer of moisture are delicate physiologic ones which will require careful study under the most exactly controlled experimental conditions before they are thoroughly understood. Eventually this phase of the problem should be carefully worked out; but at present, even though the relationship is not entirely understood, it can be said that the formation of conidiophores in both species of Philippine Sclerosporas—whether on maize, teosinte, sugar cane, sorghum, *Saccharum spontaneum*, or *Miscanthus japonicus* Anderss.—never takes place except when the surface of the host is covered with a persistent layer of moisture during the night or very early morning.

As condidiophore production in these Philippine Sclerosporas has been found by the writer to be restricted so rigidly to the night time, it is of interest to note whether any such restriction has been observed by investigators of the other important conidial Sclerosporas of the Orient. Apparently Miyake (*15*) alone appreciated that conidium production was nocturnal. Not only Butler (*3*) in his investigation of *Sclerospora maydis* (Rac.) Butl., in India, but also Raciborski (*19*), Rutgers (20) and Palm (*16*) in their successive studies of *S. Javanica* Palm, of maize in Java, failed to grasp this point. To be sure, Rutgers realized that there was a relationship between conidium production and moisture, since he stated that the fungus in the early morning was like a white down on the moist leaves while later in the day when the conidiophores and conidia were dried it gave a fine granular effect as of dried salt solution. Palm (*16*), on the other hand, regarded moisture as essential, not for the production, but for the germination, of the conidia, and consi-

dered it necessary to secure inoculating material early in the morning (around 9 o'clock) when the leaves were moist. Miyake (*15*) however, in his investigation of *S. sacchari* Miy., of sugar cane in Formosa, learned that conditions obtaining at night were in some way involved in inducing conidiophore production. In an endeavor to secure material suitable for germination experiments he placed infected cane leaves in glass jars which were inverted over shallow dishes of water. Under these conditions he found that deposition of spores always took place at night and never in the day time, no matter at what time these cultures were started. To observe the procedure more carefully he prepared similar moist chamber cultures which he examined hourly from 5 p. m. Deposition of conidia began at 1.30 a. m. and continued until 2.30 a. m. He concluded that during the day time the fungus prepares for conidiophore and spore formation which takes place during the night, but he believed his investigations needed repetition. Miyake realized that conidia were deposited at night under these laboratory conditions and inferred that this was true in the field also, but apparently he did not appreciate that this occurs repeatedly night after night on infected plants, nor did he attempt to determine whether moisture, darkness, or other night conditions are responsible.

DEVELOPMENT OF THE CONIDIOPHORES

Whenever and wherever it may occur, however, the development of the conidiophores and conidia follows the same general procedure. As has been said above, it is apparently the covering of the surface of the maize plant with moisture which influences the Sclerospora mycelium within the tissues of the host to produce conidiophores and conidia. After the host surface is thus covered, the stout mycelial branches which are located in the intercellular spaces and in the air chambers beneath the stomata (Pl. 7, G, H) begin to send out prolongations, several of which push through each partly closed stomatal pore (Pl. 7, B, C, D) and give rise to a compact group of several simple or branched knoblike processes (Pl. 7, E) which are closely appressed to the outer leaf surface, covering the stoma. From each of these knobs develops a clavate hypha (Pl. 8, B, C), the main axis of the conidiophore, which increases rapidly in size (Pl. 8, D, E) and finally forms at its apex the papillate buds (Pl. 8, J), which grow into the stout primary branches (Pl. 8, K, M). As these extend they, in turn, form at their apices the paired protrusions which become the secondary branches (Pl. 8, N); and in like manner the tertiary or even quarternary series of branches are developed (Pl. 8, O) until finally from the ultimate tips arise the sterigmata. From the apex of each sterigma buds out a conidium (Pl. 8, Q) which grows rapidly, beginning as a small, globular protrusion (Pl. 8, R, S) and passing successively in its development through globular pyriform, oval or ellipsoidal stages (Pl. 9, A–I), until finally it attains the size and shape, usually rounded cylindric, of maturity, and is cut off from the sterigma by a wall (Pl. 9, J, K, W).

These successive stages of conidiophore development require several hours for their completion. The formation of the group of knoblike conidiophore initials is not completed until the surface of the plant has been covered with moisture for two to four hours; and the development of the conidiophore and conidia to maturity consumes about three hours

more. The process is, however, a continuing one, the knoblike conidiophore initials developing into conidiophores one after another in fairly regular succession so that several stages of the transition from the initials to the completed conidiophore are usually found over a stoma (Pl. 7, F; 10, G, H).

In many cases the first "crop" of conidiophores which matures seems to be the maximum one, successive "crops" being somewhat less heavy. When badly infected leaves are examined at the time of maximum conidium production (usually about 2.30 a. m.) they are seen to present a striking appearance. The moist surface is covered with a dense grayish white "down" of innumerable conidiophores whose treelike shape can be distinguished on closer inspection.

Attempts were made to determine whether the groups of knoblike processes over the stomatal pores were renewed each night by growth from the internal mycelium, or were able, if not entirely used up in developing conidiophores during one night, to survive the day and form conidiophores the night following. In occasional instances, especially in *Saccharum spontaneum* with its sunken, protected stomata (Pl. 7, H), and during rainy or cloudy days, leaves sectioned during the day showed groups of knobs that were apparently still viable. As a rule, however, indications were that these groups dried up and were replaced by new ones which pushed out of the stoma on the next favorable night. Further investigation is necessary to settle this point conclusively.

It is noteworthy that the tips of the conidiophores customarily protrude slightly from the surface of their layer of moisture on the leaf, so that the conidia and sterigmata and perhaps even the ultimate branch tips are free, although beaded with adherent droplets of moisture or even covered by a single larger drop which involves the whole head. To some extent there is apparently a relation between the thickness of the moisture layer on the leaf surface and the height of the conidiophores. In scanty dew when the layer of moisture on the leaf is very thin, the conidiophores are short and stocky; while those which develop in deeper layers of heavy dew are usually longer. In both cases the conidia are borne just above the surface of the moisture layer. It should be noted, however, that the specific distinctions of length which exist between the conidiophores of *Sclerospora philippinensis* and *S. spontanea* are not invalidated thereby; since especially in their longer and more slender foot cells the conidiophores of the latter maintain their proportionate differences even when the two are grown under the same conditions (Pl. 8, O, P; 10, B, F).

DISCHARGE OF THE CONIDIA

In view of the fact that the conidia were borne just barely above the surface of the layer of moisture on the leaf, or even involved in adherent droplets, and yet (as will be shown later) were distributed in vast numbers by the slightest air currents, it did not seem possible to the writer that mere passive adjunction of the conidia from the sterigmata could be accountable for all that was observed. When, for instance, Petri dishes containing a thin layer of nutrient agar were placed at the base of a young, infected, corn plant from which abundant conidium production took place nightly, and when the whole was covered by a large bell jar firmly pressed into the earth and allowed to remain overnight, in the morning the surface of the agar was found covered with innumer-

able conidia. If the conidia merely dropped off the sterigmata, they would of course fall down to the Petri dishes from the lower surface of the more or less horizontal leaves; but those produced on the upper surface would hardly be carried off by the slight convection currents within the jar rapidly enough to prevent most of them from falling into the moisture on the upper surface of the leaf. Microscopic examination of the moisture on the upper and lower surfaces of the horizontal portion of such a leaf, however, showed no more spores in the former than in the latter, and relatively very few in either. Moreover, a whitish deposit of innumerable conidia often was observed on healthy leaves which, in crowded plantings, stood in close juxtaposition to infected, spore-bearing leaves. This occurred even when the parts were sheltered and were in such a vertical or oblique position as to preclude the possibility of such a deposit accumulating from conidia which merely fell.

From such observations the writer was led to suspect that the conidia were actively discharged. To obtain further information on this point the following experiments were performed on maize infected with *Sclerospora philippinensis*. During the afternoon the leaf of a badly diseased plant that was known to be producing abundant conidia each night was carefully scraped with a scalpel until its surface was entirely free from all dried remnants of the last night's conidiophores. The leaf was then fastened to a stake so that this scraped surface was held rigidly in a vertical plane. Facing this surface a slide, previously smeared lightly with clear agar, was fastened securely at a very slight angle, with the upper end almost touching the leaf and the lower end about 3 mm. away. The earth around the plant was then watered copiously; and the whole was covered with a large tin can the edges of which were pressed firmly into the earth and sealed with wet clay. Early the next morning this cover was removed carefully so as not to disturb the plant; and the slide was dexterously lifted away without touching it to the leaf surface. On examination the slide was found to be covered with freshly germinating conidia for some distance from the edge nearest the leaf. By substituting a strip of black cardboard for the slide, a faint spore-print, decreasingly dense toward the end farthest from the leaf, was obtained.

More direct evidence was secured as follows. Under a strong beam of light concentrated by a condenser, a portion of the leaf surface where abundant conidium production was taking place was watched continuously through low power magnification. Under these conditions the conidiophores, their tops heavy with clumps of maturing conidia, could be seen standing in rows corresponding to the rows of stomata below. During long observation occasional clumps of conidia were seen to flash away as momentarily gleaming specks, leaving a gap in the line of conidiophores.

In the opinion of the writer these experiments and observations indicate that the conidia are forcibly thrown off from the sterigmata. It will rest with future and more precise investigations to prove this conclusively.

Attempts were made to determine the means by which discharge of the conidia was accomplished. Because all efforts to grow the Sclerosporas in pure culture failed, it was impossible to study the developing conidiophores in Van Tieghem cells; and it was found equally impossible to adjust conidiophore-bearing leaves for study under the high power without disturbing the moisture on the leaf and interfering with conidium

discharge. However, in so far as could be determined by a study of conidiophores mounted in all stages before and after discharge of the conidia, the process apparently takes place as follows: At the point where the maturing conidium is attached to the sterigma, the wall across the base of the conidium and that across the apex of the sterigma are, when first formed, quite flat in contact with each other. Later the increasing turgidity of the conidium and sterigma causes these membranes to bulge outward, a tendency which is restrained by the adhesion of the two surfaces to each other. As this impulse to bulge outward increases more and more, however, it finally reaches a point where it overcomes the adhesion of the two surfaces; and with a sudden snap the basal septum of the conidium and the apical septum of the sterigma bulge sharply outward and shoot the conidium away. In abundant fresh material all the stages of this process can be traced, the flatness of the base wall of the conidium and the apex of the sterigma before (Pl. 9, L–V), and the bulging after discharge, being particularly noticeable.

Apparently all the conidia on one conidiophore customarily are ejected at once; but occasionally a conidiophore was encountered with most of its sterigma tips bulged out and obviously just freed from their conidia; while on one or two branches seemingly less mature, the conidia still remained in situ (Pl. 9, W).

When the conidiophore has discharged its conidia, its vitality declines, it is attacked by bacteria which swarm in the moisture on the leaf surface, and after dawn it is shrivelled by the sun to a dry, almost unrecognizable mummy (Pl. 10, C, D, E, H). In no case was seen any evidence, such as sterigmata with their apices burst open, which would indicate that an explosive ejection of the conidia, with loss of contents and subsequent collapse of the conidiophores, had taken place.

The discharge of conidia by the sudden bulging out of walls formerly flat in contact with each other was first described according to Buller (*2*) by Nowakowski for the Entomophthoreae. It has since been found by Buller (*2*) to take place in the case of the basidiospores of certain Hymenomycetes, and by Coons (*5*) also in the case of the sporidia of Gymnosporangium. In view of this, it is of interest to note that a similar method of spore discharge apparently obtains in the genus Sclerospora of a family (Peronosporaceae) in which the passive abjunction of the conidia has been assumed hitherto.

The distance which the Sclerospora conidia are ejected is very minute. With the apparatus available no accurate measurements could be made; but by such crude devices as the slanting slide mentioned above, the trajectory limit was approximately determined as about 1 to 2 mm. Small as this distance is, however, it suffices to carry the conidia into the air where they are wafted away by more or less rapid air currents; and it is noteworthy that this process of conidium discharge is remarkably well adapted to secure the dispersal by air currents of conidia flung from the moist surface of the maize plant.

NOCTURNAL CYCLE OF CONIDIOPHORE PRODUCTION

The process of conidium production which has been described follows a regular cycle under normal conditions of dew deposition. The leaves are wet with dew at 8 or 9 p. m. Groups of knoblike initials are formed over the stomata at about 11 p. m. and most of the first "crop" of conidia

has matured and is being discharged at 2 a. m. It should be emphasized, however, that this process is a continuing one; for during this time other conidiophores have been developing from the initials, so that from thenceforward a constant succession of conidia is developed and discharged. Although the maximum discharge of conidia usually takes place from 2 to 3 a. m. and lessens somewhat after that time, it does not entirely cease until the necessary moisture is dried by the early sun. This schedule applies consistently under the condition of uninterrupted dew which usually obtains during clear nights in the Philippines whether in dry, wet, or transitional seasons.

During rains, however, this schedule may be altered. When gentle rain begins in the afternoon and continues so that the plants have already been wet two or three hours by the time dew deposition begins, the formation of conidia takes place much earlier, beginning even as soon as 9 o'clock, reaching its maximum around midnight, and continuing under favorable conditions till dawn.

Observations made on May 24, 1919, furnish a good example of such a schedule. After a hot, humid day with a maximum temperature of 93°F., rain began at 4 p. m. and fell steadily for more than an hour. The sky remained clouded and the air, which was full of moisture, cooled rapidly, reaching 75° shortly after sunset and causing an early and heavy deposition of dew. At 8 p. m., when their surface had been moist uninterruptedly for 4 hours, infected maize plants were inspected. Microscopic examination showed that conidiophore production was indeed beginning, the conidiophores being in the earliest stages of development as club-shaped initials. At 9, development of the conidiophores had progressed further and the whitish down was visible under a lens. At 10, heavy conidiophore production was apparent, and quantities of conidia were matured. This production reached its maximum from about 11 until midnight but still continued, although less abundantly, at 5 the next morning, when the sun was just appearing. On plants protected from the early sun conidia were formed for about an hour more before drying. Production of mature conidia in this case continued for more than 8 hours instead of the 4 or 5 of the usual schedule.

When, after a night of abundant dew, the sky clouds over and a gentle rain begins at dawn, so that the necessary moist condition of the leaves is maintained into the morning by a constant drizzle, the process of conidium production may continue for a few hours longer. Similarly when maize is located in deep valleys or protected from the first rays of the sun by the shade of trees or hills, the dew on the infected leaves and the consequent production of conidia persists after the process has ceased on the already dried leaves of less sheltered plants.

During the dry season, also, the usual schedule may be altered. Occasionally conditions of temperature and moisture are such that dew deposition is very light and fleeting, and conidiophore production, beginning tardily, is scanty, restricted to moister parts of the plant surface and soon ceases. For example, on April 23, 1919, one of several successive dry, partly cloudy days, although the temperature, which had been 92° F. about noon, dropped gradually to 75° during the night, dew deposition was very late and scanty, as the sky was clouded and a warm, dry breeze blew fitfully. Frequent examination of infected maize plants showed that a very light dew began to gather at 10 p. m. At 3 a. m., in a thin film of dew on the leaves, a scanty production of

conidiophores was apparent. Microscopic examination showed that the first "crop" of conidia was maturing but that the conidiophores were dwarfed and scantily branched. A little after 4 a. m. conidiophore production was terminated when the dew dried as the breeze increased. In this case formation and liberation of conidia lasted for about an hour only.

The entire process of conidium production from the emergence of the internal hyphae through the stomata to the final ejection of the mature conidia takes place with remarkable regularity. Whether on infected maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, or *Miscanthus japonicus*, whether on young seedlings or senescent plants, whether on leaves, tassels, bracts, or other infected parts, the cycle of conidium production under favorable climatic conditions follows a relatively constant schedule, which may be outlined as follows:

7 to 8 p. m. Clear, still evening; dew deposition begins.

10 to 11 p. m. Plant surface wet with dew; groups of knoblike conidiophore initials are forming over the stomatal pores on the leaf surface.

11 to 12 p. m. From the knoblike bases the clavate main axes are developing and beginning to form primary branches of the conidiophores.

12 p. m. to 1 a. m. The branch system of the conidiophore is now completely developed, and conidia are beginning to bud out, while the basal cell is being cut off by a cross wall.

1 to 2 a. m. Discharge of the conidia and their dispersal by air currents begins to take place.

2 to 3 a. m. Discharge and dispersal of conidia is at its maximum.

3 to 4 a. m. Discharge of conidia lessens. Some of the first conidia discharged and scattered have reached the water held in the unrolling leaves or in the leaf axils of young susceptible plants, and there have germinated and started infection.

4 to 5 a. m. Few conidia are discharged or further conidiophores developed but germination and infection by conidia already spread continues.

5 to 6 a. m. Sun rises and soon dries the plant surfaces, killing the few conidiophores and conidia still developing from infected plants, and also the conidia which have not yet achieved the infection of the new hosts they have reached. Those conidial germ tubes, however, which have already penetrated in to the host tissue are not killed when the plant surface is dried but continue to invade the host within whose tissues they are protected.

The procedure which has just been outlined is beautifully adapted to the limitations of the delicate conidia which when dried are killed immediately. Borne on conidiophores which are submerged in dew, carried in countless numbers through the cool darkness on currents of damp air, dropped into the moisture contained by such susceptible parts as unrolling leaves, and there sending germ tubes into the succulent interior tissue of the host, the conidia, despite their delicacy, are able to accomplish infection in a remarkably efficient manner.

This procedure is also exceedingly successful in accomplishing the rapid spread of infection and distribution of the disease. Plants thus diseased will in turn begin producing conidiophores after about 10 days or, if they are young, even in 3 to 5. As a result, a small center of inoculation can under favorable conditions cause the infection of large areas in a relatively short time.

DURATION OF CONIDIOPHORE PRODUCTION

The development of conidiophores and conidia from the downy mildew mycelium ensconced within the diseased maize plant is not limited to one night, nor does it cease after it has occurred several nights, but

rather may continue to take place every favorable night for a month, six weeks, or even so great a proportion of the plant's existence as two or more months. Because this has not been realized hitherto, the danger of even a single conidium-producing plant to the surrounding fields has never been sufficiently appreciated or emphasized.

The duration of nightly conidium production was studied in a large number of infected plants, including maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, and *Miscanthus japonicus*. At first the plants were examined each night, but later the following more convenient method was used. On the badly infected leaves of each plant an area, on which formation of abundant conidiophores occurred nightly, was outlined on both the upper and lower surfaces with waterproof ink. Each afternoon these areas were carefully scraped clean from all remains of previous conidiophore production; and early the following morning the presence or absence of conidiophores and conidia showed whether the fungus had been active during the night. The lengths of the periods of nightly conidiophore production in several typical cases of infected maize are presented in Table I.

A series of such observations extending through the wet, dry, and transitional seasons brought out the following points. The length of time during which nightly production of conidia may take place on maize depends primarily on the time normally required by the host to attain maturity, on the vigor of the individual attacked, and on its age when infected. In the case of maize varieties which mature most rapidly, such as the "dwarf early" sweet and pop corns (60 to 70 days), or the Chinese or Mindanao waxy endosperm maize (70 days), the time over which nightly conidium formation may extend is relatively short, usually not over a month. In maize varieties, which mature more slowly, such as the several native flint types which are grown throughout the Philippines and other tropical places, the time is somewhat longer; while those large hardy, dent varieties and flint-dent crosses which require the longest time (3 to 4 months) to mature support the formation of conidia by the parasite for the longest period, for even more than 2 months. Apparently while the host is actively growing and developing, its internal condition is such as to favor both the growth of the parasite and the abundant production of the conidia; but when the host plant has matured, and its vitality begins to decline, its tissues apparently undergo chemical and physical changes which render them unfavorable for the further development and activity of the mildew.

The vigor of the individual plant infected also affects the duration of conidium production. Weakling plants, which result from planting imported seed of foreign varieties unsuited and unacclimatized to Philippine conditions, succumb to the downy mildew in a comparatively short time; and conidium production by the parasite, although exceedlingly abundant for a while, is soon brought to an end by the untimely death of the host. On the other hand, if strong, vigorous hardy plants are attacked they do not succumb but continue to support the persistent parasite even for as long as the lifetime of a healthy plant (Pl. 5, B). In some plants, after many weeks of abundant nocturnal spore production, conidia were still found developing on the middle and still green part of the last or next to last leaf, although all the other leaves and even the tips of these sporulating leaves themselves were drying and brown.

TABLE I.—*Duration of the conidiophore-producing period in various infected maize plants, compared with the time usually required by healthy plants of these varieties to mature*

Plant No.	Date planted.	Date emerged.	First symptoms observed.	First conidiophore production observed.	Last conidiophore production observed.	Duration of conidiophore producing period.	Time required for maturing.	Proportion of conidiophore production period to life of plant.	Number of nights conidiophore production observed during period.	Condition of plant at last conidiophore production.
						Days.	*Days.*	*Per cent.*		
MANOBO WAXY MAIZE										
13/2	Sept. 7, 1918	Sept. 10, 1918	Sept. 30, 1918	Oct. 1, 1918	Nov. 5, 1918	36	70	51.42	18	Stunted, maturing.
11/2	do	do	Oct. 8, 1918	Oct. 9, 1918	Nov. 1, 1918	24	70	34.28	13	Maturing.
1/3	do	do	do	do	Nov. 5, 1918	28	70	40.00	13	Do.
4/2	do	do	Oct. 16, 1918	Oct. 17, 1918	do	20	70	28.57	10	Do.
2/3	do	do	Oct. 17, 1918	Oct. 18, 1918	Nov. 1, 1918	15	70	21.42	7	Do.
EXTRA EARLY ADAMS SWEET										
1/1	Jan. 4, 1919	Jan. 8, 1919	Jan. 22, 1919	Jan. 24, 1919	Feb. 19, 1919	27	80	33.75	Not recorded	Stunted.
4/2	do	do	Feb. 12, 1919	Feb. 13, 1919	Feb. 27, 1919	15	80	18.75	do	Do.
GUAM WHITE FLINT										
1/4/a	Dec. 1, 1918	Dec. 6, 1918	Dec. 16, 1918	Dec. 17, 1918	Feb. 18, 1919	64	90	71.11	Not recorded	6½ feet tall with elongate ear branch.
MANGONON WHITE DENT										
2/2	Jan. 29, 1919	Feb. 2, 1919	Mar. 1, 1919	Mar. 2, 1919	May 10, 1919	70	100	70.00	Not recorded	Drying, mature.
3/1	do	do	Feb. 23, 1919	Feb. 24, 1919	do	76	100	76.00	do	Do.
3/2	do	do	Mar. 2, 1919	Mar. 3, 1919	do	69	100	69.00	do	Do.
3/3	do	do	Feb. 26, 1919	Feb. 27, 1919	April 28, 1919	61	100	61.00	do	Do.
4/2/1	do	do	Feb. 22, 1919	Feb. 23, 1919	do	65	100	65.00	do	Barren, drying, stunted.
4/2/2	do	do	Feb. 27, 1919	Feb. 28, 1919	do	60	100	60.00	do	Do.
4/3	do	do	Feb. 23, 1919	Feb. 24, 1919	do	64	100	64.00	do	Do.
4/4	do	do	Mar. 1, 1919	Mar. 2, 1919	May 10, 1919	70	100	70.00	do	Barren, stunted, slender.
5/3	do	do	Mar. 2, 1919	Mar. 3, 1919	April 28, 1919	57	100	57.00	do	Drying, barren.

KANSAS SUNFLOWER YELLOW DENT

4[illegible]	Feb. 6, 1919	Feb. 11, 1919	Feb. 24, 1919	Feb. 25, 1919	April 28, 1919	63	110	57.27	Not recorded	Barren, drying.
4[illegible]	do	do	Mar. 22, 1919	Mar. 24, 1919	do	36	110	32.72	do	Do.
4[illegible]	do	do	Mar. 5, 1919	Mar. 6, 1919	do	54	110	49.09	do	Do.

HILDRETH YELLOW DENT

3[illegible]	Feb. 6, 1919	Feb. 11, 1919	Mar. 1, 1919	Mar. 2, 1919	April 28, 1919	58	120	48.33	Not recorded	7 feet tall, tassel.
3[illegible]	do	do	Mar. 6, 1919	Mar. 7, 1919	do	53	120	44.16	do	6 feet tall, tassel.
3[illegible]	do	do	Mar. 5, 1919	Mar. 6, 1919	do	54	120	45.00	do	5 feet tall, tassel, and small ear.

The age of the plants at infection is also of importance in relation to the duration of conidium production. Plants which become infected when very young (from the time they emerge until they are a few days old) usually succumb more rapidly to the mildew than those which have had some weeks of healthy growth before they are attacked. This is shown very clearly by the observations comprised in the following summary.

In Chinese Waxy maize, planted Nov. 27, 1918, up Dec. 2, matured Mar. 3, 1919:					
Of 34 plants showing Sclerospora between	Dec. 17 and 20,	33 died between	Dec. 28 and Jan. 16,	1 survived into February.	
Of 50	do	Dec. 21 and 24, 38	do	12	do.
Of 21	do	Dec. 25 and 31, 9	do	12	do.
Of 30	do	Jan. 1 and 5, 3	do	27	do.
Of 3	do	Jan. 6 and 10, 1	do	2	do.
Of 7	do	Jan. 11 and 15, 0	do	7	do.
Of 3	do	Jan. 16 and 20, 0	do	3	do.
Of 1	do	Jan. 21 and 25, 0	do	1	do.
In Laguna Mexican maize, planted Nov. 29, 1918, up Dec. 11, matured Mar. 20, 1919:					
Of 34 plants showing Sclerospora between	Dec. 17 and 21,	34 died between	Dec. 28 and Jan. 16,	0 survived into February.	
Of 11	do	Dec. 22 and 26, 9	do	2	do.
Of 5	do	Dec. 27 and 31, 3	do	2	do.
Of 20	do	Jan. 1 and 5, 10	do	10	do.
Of 10	do	Jan. 6 and 10, 2	do	8	do.
Of 4	do	Jan. 11 and 16, 0	do	4	do.
In Kansas Sunflower Yellow Dent, planted Feb. 6, 1919, up Feb. 11, matured May 30, 1919:					
Of 11 plants showing Sclerospora between	Feb. 25 and 28,	10 died between	Mar. 25 and Apr. 15,	1 survived into May.	
Of 1	do	Mar. 1 and 5, 0	do	1	do.
Of 1	do	Mar. 6 and 25, 0	do	1	do.
In Mangonon White Dent, planted Jan. 29, 1919, up Feb. 2, matured May 10, 1919:					
Of 8 plants showing Sclerospora between	Feb. 16 and 20,	8 died between	Mar. 1 and Mar. 25,	0 survived into May.	
Of 11	do	Feb. 21 and 24, 8	do	3	do.
Of 9	do	Feb. 25 and 28, 7	do	2	do.
Of 4	do	Mar. 1 and 4, 0	do	4	do.
Of 1	do	Mar. 5 and 25, 0	do	1	do.
In Yucatan pop corn, planted Dec. 3, 1918, up Dec. 10, matured Mar. 31, 1919:					
Of 16 plants showing Sclerospora between	Dec. 19 and 24,	14 died between	Dec. 28 and Jan. 10,	2 survived into February.	
Of 23	do	Dec. 25 and 31, 19	do	4	do.
Of 3	do	Jan. 1 and 4, 0	do	3	do.

Moreover, such early-infected plants are usually much more liable to the destructive attacks of such secondary parasites as Pythium, Fusarium, Helminthosporium, and bacteria.

Occasionally the period of conidium production is prolonged by the abnormal growth of the diseased plant. In response to some stimulus exerted by the parasite, various parts of the host, especially the shank, husks, ear, and tassel, may develop abnormally, the activity of the mycelium keeping pace with this unduly extended growth of the plant body. In one striking case of this sort, a plant of Guam maize (Pl. 5, B) which showed symptoms of infection after vigorous growth had well begun, supported nightly conidium production by the downy mildew from the time it was 17 days old during its subsequent development. After about 5 weeks, when a normal tassel had been produced, the very young ear began to develop; and, abnormally stimulated by the downy mildew mycelium which had invaded the bud as it formed, the shank grew excessively in length, forming a branch which was clothed with extensive leafy husks, almost equaled the main axis in length, and bore at its tip a small sterile ear. The remarkable growth of this abnormal ear branch occupied about 30 days. The branch was still green and vigorous long after the healthy plants nearby were dry, and about 14 days after the upper part of the main axis had begun to wither. During all this time nightly conidium formation by the parasite was taking place from all parts of the excessively developed leafy husks of the abnormal branch, thus prolonging the period of conidium production by the infected plant over a total of more than nine weeks. Finally, about two weeks after conidium formation was observed for the last time, the vitality of the branch declined and it gradually withered.

The behavior of the downy mildew on its other hosts presents an interesting comparison to that which is characteristic for maize. In the few sorghum plants that became infected, the period of conidium production was necessarily brief, as death occurred a few weeks after infection. This was true also in the only cases of conidially infected sugar cane which were encountered (*25*). Moreover, on both these hosts the nocturnal conidium production during this brief period was far less extensive and abundant than in maize. In teosinte, on the contrary, the duration of conidiophore production is prolonged beyond that of maize, because of the constant renewal of young tissue suitable for spore production. As new shoots are sent out from the base of the infected plant (Pl. 6, A, C), or as new branches arise, the fungus mycelium of the main axis grows out into the freshly formed tissue, keeping pace in its development with the renewed growth of the plant, on which it produces additional conidiophores. In this way conidium production, although not so abundant on any one night as in maize of equal age, is prolonged and continues constantly and persistently for several months. This was also true in teosinte-maize hybrids, which showed extensive growth of numerous suckers and branches. In *Miscanthus japonicus* the period of conidium production was similarly prolonged. It is in *Saccharum spontaneum*, however, that this prolongation is greatest. Infected plants kept under observation were still producing conidia on many newly formed shoots even after an uninterrupted period of over eight months (*25*). Moreover, conidium production was renewed on new shoots arising from the well-established rootstocks of infected plants even

after the plants either when young or when quite passé were cut back to the ground surface. Thus conidium formation by the parasite on *Saccharum spontaneum* can be as persistently perennial as the grass itself.

QUANTITY OF CONIDIUM PRODUCTION

The number of conidia produced from a single maize plant infected with downy mildew even during one night is exceedingly great, while the total conidium production from such a plant throughout the entire season is appallingly vast. It is necessary to consider this matter further in order to appreciate the infectious menace to a maize-growing district which is embodied in even a single diseased plant. The amount of conidium production from any one plant during any one night depends on three factors: First, the extent on the host of the discolored area which is most heavily invaded with mycelium and especially active in conidiophore production; second, the number of stomata in both surfaces of this area; and third, the proper environmental conditions to induce conidium formation.

The number of conidia borne by a plant is directly proportional to the extent of the yellowed, whitened, or otherwise symptomatically discolored part of its surface. Although the hyphae of the parasite are to some extent found in and may give rise to conidiophores on the apparently unaffected parts of the host, it is from the discolored areas which have become etiolated through the destructive invasion of abundant mycelium of the fungus that the great mass of conidiophores emerges. The development and progressive increase in extent of this abundantly conidiophore-bearing surface has been studied both in inoculated plants grown under controlled conditions and in naturally infected plants in the field. The procedure is as follows: After the young plant is infected, there ensues a latent period of from a few days to more than two weeks, its length being directly proportional to the age of the plant when inoculated. The mycelium spreads throughout the plant tissues and concentrates especially in those areas of the leaves and leaf sheaths which begin to show symptomatic etiolation. The production of conidiophores on the whitened areas then commences. At first the conidiophores appear scatteringly (Pl. 1, A; 4, A, B), but production gradually increases during subsequent nights until the affected areas are covered with a dense down of conidiophores (Pl. 4, C, D). In proportion as new leaves unroll from the bud, and as the leaves, which were already partly developed when conidium production began, continue to elongate by intercalary growth, the conidiophore-bearing surface is constantly augmented (Pl. 1, C). Finally, when all the leaves are fully developed, the production of conidiophores reaches its maximum. Even then, in badly infected cases, this production may be still further increased by the formation of conidia from the tassel, from the husks, which are often abnormally developed (Pl. 5, B), and from the peculiar bractlike structures which frequently form in abnormal tassels (Pl. 5, A) or around abortive ears. Thus the conidiophore-bearing surface, which may be established when the infected plant is only about a week old, and which at first may be only a few square centimeters in extent (Pl. 2, A; 3, A), gradually increases in area (Pl. 2, B; 3, B) until it attains its maximum of some two or three thousand square centimeters; and then, decreasing in extent as successive

leaves of the maturing host wither, it finally ceases to support conidium production after months of activity. The extent of the discolored conidium-bearing areas on plants of various ages has been determined for the different maize types most common in the Philippines. The least area is found in the case of such small, rapidly maturing varieties as Manobo and Davao Waxy Maize and the dwarf native flints. The maximum area occurs in such large, slowly maturing types as introduced American dent varieties or those strains, such as "Moro" White, developed from dent-flint crosses. Between these two extremes lie the intermediate areas which are typical of the medium-sized, moderately rapidly maturing yellow and white flint types that are so commonly grown throughout the islands.

In order to gain an idea of the extent of the conidium-producing areas of diseased plants under the usual field conditions, measurements were made of characteristically infected specimens both of the common native-grown maize and of the introduced types under cultivation at the college. Some of these measurements are shown in Table II. In obtaining these data the following method was used: Leaves bearing conidiophore-producing areas were carefully removed from infected plants and pinned down to large sheets of paper upon which the outline of the whitened areas was carefully traced through the tissue of the leaf with a sharp stylus. The space inclosed within these tracings was then computed by means of a polar planimeter. Only portions which were found by actual observations to be producing conidiophores were included. In the specimens tabulated, therefore, the measurements give a fair idea of the amount of surface on which conidiophores were borne. The extent and general configuration of this surface on the successive leaves of two of the plants included in Table II are shown in Figure 1.

To the second factor, the number of stomata in the conidium-bearing surface, the quantity of conidia produced is also directly proportional. In no case were conidiophores developed except from the stomata, and in the manner already described. The stomatal frequencies recorded by Eckerson (*8*), Duggar (*7*), Kiesselbach (*13*), and others show a wide range of variation not only between different individuals or varieties under similar or diverse environmental conditions but also between different parts of the same plant. In the present instance, therefore, no extensive counts were made, but enough material was examined to determine that in the principal types of infected maize studied in the Philippines the stomatal frequencies fall easily within this range. In such varieties as Manobo Waxy, the number agreed closely with Kiesselbach's counts for Chinese Waxy; while in the large flint-dent crosses, such as Moro White, the number closely approximated his counts for various dent types. In general, it was found that his averages of 77 and 93 per square mm. of upper and lower surface were sufficiently conservative to serve as fair mean values for calculation in the Philippine maize varieties studied.

TABLE II.—*Conidiophore-bearing areas on representative maize plants of various ages infected by downy mildew*

No.	Approximate age.	Number of leaves.	Approximate height.	Whole area.			Conidiophore-bearing area.			Percentage conidiophore-bearing.	Conidium production.
				Blades.	Sheaths.	Total.	Blades.	Sheaths.	Total.		
	NATIVE YELLOW FLINT										
	Weeks.		*Cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*		
1	2 to 3	6, seventh unfolding	67.0	786.00	144.50	930.50	372.70	3.74	376.44	40.45	Very heavy.
2	4	7, eighth unfolding	84.0	1,130.64	258.84	1,389.48	551.40	42.00	593.40	42.70	Do.
3	4	9, tenth unfolding	93.0	1,402.15	369.66	1,771.81	589.04	31.42	620.46	35.01	Medium.
4	10	8, and tassel	101.0	1,409.07	426.50	1,835.57	723.30	71.56	794.86	43.30	Heavy (stunted plant).
5	4 to 5	8, ninth unfolding	91.0	1,819.02	349.15	2,168.17	351.70	39.77	391.47	18.05	Very heavy.
6	7	9, tenth unfolding	115.0	2,052.50	392.68	2,445.18	835.00	12.54	847.54	34.66	Do.
7	10	9, and tassel	122.0	2,154.33	539.71	2,694.04	1,145.45	98.93	1,244.38	46.19	Do.
8	7 to 8	11	120.5	3,166.47	520.66	3,687.13	851.60	22.08	873.68	23.69	Medium.
9	8 to 9	12, and tassel	126.0	4,112.09	790.35	4,902.44	1,146.05	161.16	1,607.21	32.78	Heavy.
10	10	.....do	192.0	5,127.90	1,371.70	6,499.60	2,629.20	571.08	3,200.28	49.23	Very heavy (full-sized plant).
	MEXICAN JUNE.										
11	10 to 11	9, and tassel	126.0	1,502.78	429.92	1,932.70	1,346.55	96.99	1,443.54	74.69	Medium.
12	6 to 7	9	115.0	2,268.68	352.15	2,620.83	1,049.30	12.45	1,061.75	40.51	Very heavy.
13	7 to 8	9, and tassel	136.0	2,193.61	323.97	2,517.58	1,001.25	173.44	1,174.69	46.65	Heavy.
14	7	9, tenth unfolding	135.0	3,228.39	530.30	3,758.69	1,511.40	30.60	1,542.00	41.02	Very heavy.
15	8	11	138.0	3,220.19	855.22	4,084.40	1,934.48	470.71	2,405.19	58.88	Do.
16	9 to 10	9, and tassel	154.0	3,656.79	926.32	4,583.11	1,285.80	114.84	1,400.64	30.56	Medium.

The third factor, environmental conditions favorable for conidium formation, bears on the quantity of conidia produced as follows: Under the usual field conditions, when the infected plants have begun to show the characteristically discolored areas, and these have begun to bear conidia, the number borne is at first comparatively small, as the conidiophores are few and scattered. As time goes on, however, and conidiophore production is induced night after night successively, production rapidly increases until on the area already established it attains its maximum density. This maximum density under such favorable environmental circumstances may occur even before this area has reached its maximum size. On the contrary, if such a plant has been exposed to conditions preventing conidiophore production, the area, even though long established, will not produce large numbers of conidiophores until favorable conditions have prevailed for so long a time that conidium production has been enabled to build itself up to the maximum.

It is apparent, therefore, that a plant to produce a maximum quantity of conidiophores must have: First, a large conidiophore-bearing surface; second, a large number of stomata per unit of this surface; and third, continued favorable conditions which permit conidiophore production to increase night after night to its maximum. These requirements are fulfilled in such typically Philippine varieties as Moro White, Native Yellow, and Cebu White, or in the even larger introduced dent and flint types. After studying conidiophore production on such varieties at night, and finding plant after plant with the greater part of its leaf surface white with a felt of densely grouped conidiophores (Pl. 4, C, D), and after seeing the thousands of spores which fill even a minute droplet scraped from the surface of such a leaf, one is impressed by the vast production of conidia which goes on. To get a more definite conception of this vast production the writer made approximate estimates of the number of conidia formed on typical plants. These estimates were calculated as follows: The total surface bearing conidiophores was multiplied by the number of bearing stomata in a unit of surface, and this result by the average number of conidiophores from each stoma, and this in turn by the average number of conidia per conidiophore. For the conidiophore-bearing surfaces the measurements recorded in Table II were used. For the number of stomata the averages of Kiesselbach were employed, but as microscopic examination showed that usually only about one-half the stomata actually bore conidiophores the averages, 77 above and 93 below per square millimeter of surface, were reduced accordingly. The number of conidiophores bearing conidia at each stoma when examined at different times during the night was found to vary from one to four, with two as a conservative average. From the number of club-shaped initials in various stages of growth at each stoma the number of conidiophores successively developed per stoma during the nightly cycle was approximately estimated as six or three successive "crops" of two each. The number of conidia on the conidiophores, although very variable, most commonly ranges from 32 to 48; 40, therefore, was selected as a fair average. The results of the calculations are shown in Table III.

These figures may serve to give a clearer idea of the amount of conidium production on a diseased plant than can be obtained merely from description. They are, of course, only approximate; but they are extremely conservative, and purposely have been made to underestimate rather than overestimate the number. Obviously a single diseased plant, even a

small one and one of relatively low spore production, is able to develop dangerously large quantities of conidia each night; while a large plant with maximum spore formation in its nocturnal cycle liberates a startlingly vast number of conidia. From extensive, badly infested maize fields in which 50, 70, or even more than 80 per cent of the plants are heavily conidiophore-bearing, an incredibly large number of conidia is produced. Moreover, it should not be forgotten that this production continues night after night for months, thus achieving a total output of conidia that is almost unbelievable. With the magnitude of this spore production in mind it is easy to understand the rapid spread of the downy mildew under Philippine conditions from even small isolated centers of infection, and to appreciate the menace to the whole maize-growing countryside which one infected field or even one single badly diseased plant may represent.

TABLE III.—*Calculation of approximate number of conidia liberated from the conidiophore-bearing surfaces of representative maize plants during one night*

Plant No.	Conidiophore-bearing surface.			Average number of stomata per square centimeter.	Average number of maturing conidiophores per stoma at one time.	Average number of conidia per conidiophore.	Number of conidia produced on plant at one time.	Successive "crops" of conidiophores produced during night (average).	Total number of conidia produced on plant during night.
	Leaf surface (2×area).	Sheath area.	Total.						
	Sq. cm.	*Sq. cm.*	*Sq. cm.*						
NATIVE YELLOW									
5	703.40	39.77	743.17	4,250	2	40	252,677,800	3	758,033,400
6	1,670.00	12.54	1,682.54	4,250	2	40	572,063,600	3	1,716,190,800
7	2,290.90	98.93	2,389.83	4,250	2	40	812,542,200	3	2,438,126,600
9	2,292.10	161.16	2,453.26	4,250	2	40	834,108,400	3	2,502,325,200
10	5,258.40	571.08	5,829.48	4,250	2	40	1,982,023,200	3	5,946,069,600
MEXICAN JUNE									
12	2,098.60	12.45	2,111.05	4,250	2	40	717,757,000	3	2,153,271,000
14	3,022.80	30.60	3,053.40	4,250	2	40	1,038,156,000	3	3,114,468,000
15	3,868.96	470.71	4,339.67	4,250	2	40	1,475,487,800	3	4,426,463,400

DISPERSAL OF CONIDIA

When the conidia are being produced their distribution, and the resulting spread of the downy mildew, is accomplished primarily by wind, to a less extent by splashing or wind-blown rain, and to a still less extent by surface water. Obviously, distribution of effective, germinable conidia can take place only at night or possibly, under unusually favorable conditions, until a little after dawn, because the conidia can not survive drying. Obviously, also, any conditions restricting or favoring conidium production in like manner directly affect their dispersal.

DISPERSAL BY WIND

The two Philippine Sclerosporas of maize are admirably adapted to distribution by wind as their conidia are produced in vast numbers and are sufficiently small so that they may be readily carried in the air. The great number of conidia produced has already been considered. The size of the individual conidium, although far larger than that which

characterizes the breeze-borne basidiospores of the Hymenomycetes and slightly larger than that of the wind-carried oidia of the oak mildew, is relatively small, being far less than that of the air-distributed spores of Lycopodium, or of the pollen of anemophilous conifers and grasses. From its dimensions the volume of an average Sclerospora conidium may be calculated roughly as follows: If the spore is regarded as consisting of a cylindrical main portion with hemispherical ends, as is diagrammatically shown in figure 2, the total volume in cubic μ will equal the volume of this cylinder plus that of the two hemispheres. In *Sclerospora philippinensis*, the most commonly encountered length is 33 to 35 μ (34 μ), the most frequent diameter 17 to 19 μ (18 μ); while the greatest length is 52 μ, and the greatest diameter 24 μ. On the basis of these measurements we may calculate the most commonly encountered volume as 7,125.15 cu. μ (0.000,000,007,125 cc.), while the greatest volume is similarly found to be 19,905.18 cu. μ (0.000,000,019,905 cc.). In like manner, in *S. spontanea*, the most commonly encountered length is 42 μ and the diameter is 16 μ, while the greatest length is 64 μ and the greatest diameter 20 μ, giving a most common volume of 7,372.29 cu. μ (0.000,000,007,372 cc.), and a greatest volume of 18,011.84 cu. μ (0.000,000,018,011 cc.).

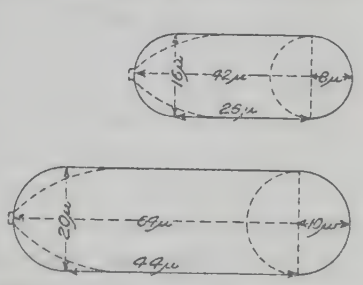

FIG. 2.—Diagrams representing *Sclerospora spontanea* conidia of the most common and of the largest size schematized to facilitate calculation of approximate volumes.

The specific gravity of the conidia was not determined. It was observed, however, that the spores, although they occasionally float, usually sink very gradually in a water mount. It may probably be assumed without serious error that their specific gravity is little if any greater than that of the heaviest Hymenomycete spores (*Coprinus plicatilis*, 1.43) studied by Buller (*2*). Their weight, therefore, is relatively very small.

The rate of fall of the Sclerospora conidia was not determined. The fall of these conidia, because of their larger size, would undoubtedly be faster than the velocity of 0.429 cm. a second, found by Buller for Coprinus; but it would not, in all probability, exceed markedly the 1.76 cm. a second determined by Zeleny and McKeehan (*26*) for Lycopodium spores which have a specific gravity of 1.175 and a diameter of 31.6 μ. Furthermore, because Buller found that the elongate spores of *Polyporus squamosus* when falling soon assumed a position with the long axis horizontal and thus were retarded by the increased air resistance, it is probable that the similarly shaped Sclerospora conidia, even though larger, behave in like manner. In any case it seems justifiable to assume that the rate of fall of the conidia is sufficiently slow to facilitate their being carried by breezes of even slight strength. Judging from the foregoing, conidia, even in absolutely still air, when shed from the upper leaves of maize plants at a height of 1.76 meters would require nearly three minutes (176 seconds) to reach the ground, and that from a height of 3.52 meters, which is not infrequently attained by conidiophore-bearing tassels and topmost leaves of large maize varieties, the conidia would take but little less than six minutes to complete their fall.

Moreover, Schmidt (*21*) shows that widespread distribution of a large proportion of liberated spores is accomplished by winds of low velocity. He calculates that Lycopodium spores which have a rate of fall of 1.76 cm. a second, when liberated in a wind blowing 36 kilometers (22.5 miles)

an hour possess an average limit of distribution of 330 kilometers (206.25 miles). Similarly even in the case of *Pinus sylvestris* pollen, which has as great a diameter as 48 μ, and as rapid a fall as 5.3 cm. a second, he computes that as many as four-tenths of the number originally shed would be carried 4 kilometers by a wind of 36 kilometers an hour; while two-tenths would reach 13 kilometers; one-tenth would reach 20 kilometers; and one-hundredth would reach 36 kilometers. The work of Hesselman (*12*) shows that these calculations represent the conditions attained in nature. He found that conifer pollen grains 30 to 60 μ in diameter were deposited in relatively vast numbers in dishes on light ships 30 kilometers (18.6 miles) and 55 kilometers (34 miles) from land; while pollen of grasses also, in spite of being liberated in far less abundance, nearer the earth, and unequipped with appendages for buoyancy, was caught in considerable quantities.

It seems justifiable to conclude from the foregoing evidence that the conidia of Sclerospora also are easily and effectively distributed by air currents. The writer's observations also bear this out. Small, infected maize plants, known to be producing abundantly, were covered at dusk with large cans or bell jars the edges of which were pressed into the earth and sealed with clay. The nocturnal fall of innumerable conidia then took place undisturbed except by such slight convection currents as were operative within the can. As a result Petri dishes placed on the ground under the plant, or, even the ground itself, showed a heavy deposit of spores which in some cases was sufficient to whiten the earth conspicuously. When, however, the plants were left exposed to the wind, no such abundant spore deposit was found under them even after apparently calm nights; and after nights with gusty winds almost all the conidia were carried away, few, if any, being caught in the exposed Petri dishes under the plant. Further observations and experiments concerning the distribution of conidia by winds of different velocities will be given in the following sections.

The dispersal of the conidia by wind is of three main types; first, by very slight local air currents; second, by more general breezes of greater strength; and third, by violent and extensive typhoons.

BY SLIGHT AIR CURRENTS

The slight air currents, although very gentle and necessarily restricted in extent, are very important, because of their invariable occurrence, in distributing the conidia locally. Even during nights when the air is apparently absolutely still, careful scrutiny will show that here and there a corn leaf is moving slightly in air currents otherwise imperceptible. These nightly driftings of the air are necessarily confined to restricted areas and are influenced in direction and extent by very local conditions. At the College of Agriculture of the Philippines where, for the most part, the dispersal of conidia was studied, the influence of Mount Makiling was the most noticeable factor. By this heavily forested mountain, which rises to a height of over 3,300 feet, the air is cooled at night and flows down the slopes to the plain. The presence and direction of this air flow were determined under varying night conditions by repeatedly observing the course and velocity of buoyant seeds, such as those of Ceiba or Saccharum, which were thrown into the air and followed by the beam of an electric torch. Field observa-

tions showed that the spread of the downy mildew in the corn plantings closely followed this air flow in direction and extent. Plots containing recently germinated and extremely susceptible maize seedlings developed few if any cases of the downy mildew when located toward the mountain to windward of actively infective plants. Similar plots, however, situated away from the mountain to the leeward of the same actively infective plants and in the direct path of the stream of conidia borne on the nightly air currents from them developed a very high percentage of downy mildew cases.

In larger fields it was possible also to demonstrate that the spread of the downy mildew closely followed the direction and extent of the nightly air currents. When, after carefully removing any actively infective plants so situated as to be dangerous to such a field, a single conidium-producing plant was transplanted to the center of it, that portion of the field located to windward of the diseased plant remained free from disease except in the very immediate vicinity of the plant itself. In the leeward part of the field, however, as the young corn developed, there subsequently appeared numerous cases of downy mildew. These were especially frequent along the fan-shaped path of the conidia which were carried by the nightly air currents from the centrally placed diseased plant and were increasingly numerous the nearer one approached the source of infection. Although, as has been mentioned, the general trend of these nightly air currents was down from the mountain, it was found by experimenting that their extent and direction were greatly influenced by local conditions. Such topographical features as wooded ridges, hills, deep gullies or brook beds, and small bodies of water had a marked effect; while buildings, thick woods, and dense growths of tall cogonal grasses often served also to obstruct or deflect the air currents. Even trees, bamboo or banana clumps, and small plots of sugar cane, sorghum, or cassava were found to disturb the nightly air currents by causing little eddies and local circulations of air.

Petri plates containing a thin film of clear 1 per cent agar were employed to gain a more exact idea of the direction and extent of conidium dispersal by these nightly air currents. On an apparently still night such plates were exposed during the period of maximum conidium formation at various distances around a single diseased plant which was producing abundant conidia. All other actively infective plants in the vicinity having been removed, careful record was made both of the position of the plates and of the direction of the prevailing air currents in relation to the diseased plant. At dawn the plates were carefully examined under the microscope and the presence and number of conidia noted. Very few spores were found in plates situated to windward of the infective plant, and only in those which had been very near it (within 3 or 4 feet). To the leeward of the infective plant, however, and directly in the path of spore distribution especially, large numbers of conidia were caught. These were most numerous in plates near the plant and proportionately less abundant in those at greater distances. Plates within 8 or 10 feet were liberally showered; those 20 and 30 feet distant showed many conidia; while those situated 50, 60, and in one case, even 80 feet away still caught a few. As the Petri dishes were of small size (10 cm. diameter) it was to be expected that at greater distances the conidia would be so scattered as to reduce to the minimum the chances of their being captured. It should be noted that the conidia whether caught at 8 or 80 feet germinated vigorously on the moist agar surface.

BY BREEZES OF GREATER STRENGTH

Dispersal of conidia is accomplished also by breezes and night winds of moderate strength. By these the conidia are carried more rapidly and to a far greater distance than by the gentle, almost imperceptible air currents just considered. At the College of Agriculture at Los Banos stronger winds and breezes play a lesser part in the total conidium distribution than does the nightly air drift, because they are relatively infrequent, and often occur when storms are in the vicinity and when the sky is cloudy so that no conidia are produced. Occasionally, however, gusts of brief duration but of considerable strength were noticed during nights when abundant spore production was taking place. These were most noticeable just before dawn. Spore dispersal is most effectively accomplished when the breeze comes in such occasional intermittent puffs, as when constant it tends to dry the dew from leaf surfaces and thus kill the conidiophores that are being produced.

In certain localities, where conditions are more favorable, stronger breezes occur with great regularity when conidia are being formed. In regions near the coast the on-shore breeze dies down after sunset; and as the earth cools, an off-shore breeze springs up, starting at a considerable distance inland, and blowing toward the sea. This was particularly noticed in the vicinity of Lingayen Gulf, Pangasinan Province, where a distinct off-shore breeze was found to prevail at night, sweeping from far inland over the maize fields toward the shore. In extensive flat plains also, noticeable breezes arise at night and sweep over considerable areas even on nights when sufficient dew formation takes place to insure the abundant formation of conidia. This was found to be the case in the broad plain which runs from Batangas and Balayan Bays northwest through Batangas and Laguna Provinces to the Lake of Bay. Throughout this plain where corn occupies about 31,000 acres, a large proportion of the cultivated land, and where the downy mildew is exceedingly destructive, such breezes were found to be a very important factor in the distribution of the downy mildew. Observations were made at Taal in the midregion of this plain, at Calamba, near the northern limit, and at Batangas on the southern coast; and in each place marked breezes were noted on nights when conidium production was occurring. At Batangas the off-shore breeze to the bay also influenced the spore dispersal, while at Taal and Calamba the effect of the presence of the lakes was noticeable.

BY VIOLENT GALES

Dispersal of conidia is accomplished by night winds of high velocity and great strength, such as those occurring during severe tropical storms. At first it seemed doubtful to the writer if conidium production could take place under such conditions even though leaves were moist. Accordingly, plants known to be producing abundant conidia on normal dewy nights were observed at night during storms of varyin degrees of violence. These observations showed that, on the whole, conditions which prevailed during such storms were unfavorable to conidium formation. Frequently the torrents of driving rain and the terrific gusts of wind lashed the leaves about so violently, or even beat down the whole plant so completely into the mud, that the delicate conidiophores were prevented from developing. During less violent storms, the rain, although sufficiently gentle in itself to permit conidium formation, was

frequently intermittent and alternated with gusts of wind which blew the water from the leaves and dried the developing conidiophores sufficiently to kill them. None the less conidium production could and did take place during these tropical storms despite their unfavorable violence. In all but the most destructive typhoons at least a few plants in each plot were sufficiently sheltered from mechanical injury so that their leaf sheaths, the under sides of midribs at the base of the leaves, or other protected parts, permitted the production of considerable numbers of conidia. In some cases conidium formation actually took place on the inner surface of leaf sheaths (Pl. 4, E), husks, or bracts which were so situated that they shed direct beating rain. Moreover, the storms frequently began with a cold rain which fell steadily for from one to about three days, and the wind, when it arrived, came first in intermittent gusts before increasing to its full strength. Under these conditions conidia were produced in abundance on large numbers of plants and for several hours were given ample opportunity to be distributed by the wind before its violence was sufficient to injure the developing conidiophores.

As a concrete example of conidium production under unfavorable storm conditions observations made in the typhoon of December 25 and 26, 1918, are of interest. This storm was an unseasonable one, occurring at the beginning of the dry season, and preceded as well as followed by calm, dry weather. It was also a violent one. During December 25 and 26 the rainfall was 59 mm. and the wind, by anemometer, blew 262.8 miles, while during the rest of the month the rainfall averaged 1.17 mm. and the wind 62.2 miles, a day. Cloudy, sultry, threatening weather immediately preceded the storm which began in the afternoon of December 25 with occasional gusts of wind and squalls of rain. The temperature, which during the hottest part of the day, had reached 82° F., decreased rapidly, dropping after sunset to 70°. The plants under observation were well covered with moisture despite the increasing violence of the storm. By 11 o'clock that night the rain fell heavily and almost continually, while the wind was violent but intermittent. From 1 to 2.30 a.m., December 26, the storm raged at its height with rain and wind of such terrific force that examination of the plants was impracticable. After this, however, a gradual decrease was apparent, and by 4 a.m. the rain and wind had lessened, although violent squalls occurred from time to time. The maize plots were then examined. Many plants, both healthy and diseased, had been blown flat and beaten into the wet earth. However, several heavily infected plants which had been under observation showed conidiophore production in abundance on the better protected parts of their leaves and sheaths. Microscopic examination showed many conidiophores which had already discharged their conidia, hence production had probably been going on for about two hours and even during the worst of the storm. Formation of conidia continued until about 6 a.m. when it was checked as the plants began to dry. During this time the wind continued to blow in gusts favorable for spore dispersal. By afternoon the storm had disappeared.

It was, of course, impracticable to determine the precise amount or extent of dispersal of conidia during storms by any such direct means as Petri dishes. Observations in the field, however, showed repeatedly that in regions where the disease had been spreading slowly on the slight nightly air currents, a sudden scattering of the disease appeared, after the normal incubation period following the storm, for several miles along the track of the typhoon.

Wind, in so far as the writer was able to determine, is the most important factor in scattering the conidia of the Philippine maize mildews. In the other cereal downy mildews of the Orient various methods of conidium dispersal have been emphasized by previous investigators. Unfortunately, Butler's (*3*) discussion of the downy mildew of maize in India does not consider this point. With regard to the Sclerospora of corn and sugar cane in Formosa, Miyake (*15*) states that he obtained no direct experimental data on the methods of spread of the conidia in the field because he could not collect spores with germinating power. From field observations, however, he concludes that the spores are distributed by wind, by rain, and by irrigation water; but he regards the distribution of diseased cuttings of cane as the most dangerous and effective method of spread. In the case of the Javan maize mildew, Rutgers (*20*) disregards the matter of conidium dispersal and considers it doubtful if infection by conidia occurs normally in the field in spite of the fact that Raciborski (*19*) had secured infection with conidia and had concluded that they undoubtedly were spread by the wind. Palm (*16*), however, rightly infers from field observations that the disease spreads by the wind dispersal of conidia, and he fully appreciates the importance of this method. It should be noted, however, that his experimental evidence (the finding of conidia in Petri dishes exposed in the path of the wind 10 to 50 meters from a 60 per cent infected field) is valueless. As these plates were exposed during the day and "Het weer was zonnig en droog," the conidia caught were undoubtedly the dried remainder from a previous night's crop, quite dead, and quite incapable of germination or infection. By his experiments "the presence of the conidia in the air was shown" as he says (in translation), but as these conidia were incapable of germination their presence in the air gave no proof of their ability, wind-borne through they were, to spread infection. It would be analogous to reason that certain species of grasses are distributed by ocean currents because the seed, although killed by immersion in salt water, are carried long distances in the sea.

SPLASH DISPERSAL

Dispersal of the conidia is also accomplished by splashing. On dewy nights this is constantly taking place as the large drops of accumulating dew drip from the upper leaves, strike the conidiophore-bearing surfaces of the leaves below and splash off, carrying innumerable conidia to neighboring plants. Droplets thus splashed off, when caught on sheets of glass and examined with the microscope, were found to contain large numbers of conidia which germinated vigorously in dew or on agar. Although the spreading of infection undoubtedly occurs in this direct way, it is of comparatively limited extent and involves only plants in the immediate vicinity. During rains, however, provided conidium production is taking place, the conidia are splashed far greater distances; and, when the splashed-off droplets are carried by the wind, whether by the gentle air currents of still dewy nights or by the gales of violent tropical storms, the distance of dispersal is greatly and correspondingly increased.

The distance over which such droplets are spread either by simple splashing after falling from various heights and upon different kinds of surfaces or by spattering into and being carried away by air currents of various strengths has been studied very thoroughly by Faulwetter (*10*).

From his investigations it seems safe to assume that in the downy mildews of maize splash distribution would be accomplished as follows: Drops of dew dripping upon moist spore-bearing leaf surface only 12 inches below would scatter droplets full of conidia over an area 20 to 32 inches in diameter. Raindrops falling from a height of only 16 feet upon such leaf surfaces 4 feet above ground would spatter droplets containing conidia for a distance of 3 feet 2 inches if the leaf was level or as much as 5½ feet if the leaf surface inclined 30°. Droplets spattered thus from a leaf 4 feet above the ground would be carried as much as 18 feet by a wind blowing 10 miles an hour and proportionately farther by winds of greater velocity. Also conidium-containing droplets would be relayed on from plant to plant by splashing and by wind throughout the duration of a storm. Faulwetter (*9*) demonstrated that spattering and wind-blown rain were important factors in the dissemination of the angular leafspot of cotton. The writer, from a study of the maize downy mildews of the Philippines, is convinced that these factors are operative in their distribution also.

The two methods thus far considered—namely, wind distribution and splash distribution acting either separately or together—are the main instruments in achieving dispersal of conidia of the maize downy mildews under Philippine conditions.

DISPERSAL BY AGENTS OF MINOR IMPORTANCE

Surface water apparently accomplishes conidium dispersal to some extent by washing the conidia which fall in it into contact with young seedlings which are temporarily submerged. Although this was not shown to take place by direct experiment, nevertheless there is considerable evidence of infection occurring in this manner. When glass dishes containing brook water and rain water were exposed at nights under plants producing large numbers of conidiophores, great quantities of conidia were caught. Some of these floated while others sank, but all germinated vigorously. When these dishes were exposed to the sun the conidia were soon killed, probably by the rapid increase in the temperature of the water. When the dishes were protected from the sun, however, the conidia retained their vitality and continued to germinate and grow until some hours after dawn. Young, susceptible plants, inoculated with a concentrated suspension of living conidia from these dishes, soon developed the disease. Moreover, in the field it was frequently noticed that during the rainy season low-lying plots often became submerged under a few inches of standing surface water at a time when the maize had just germinated and the young seedlings were in their most susceptible stage. Extremely heavy infection generally resulted in these inundated areas, the remainder of the field being much less affected. This was also observed by Baker (*1*) who wrote:

> In one interesting case, a part of a field had been subjected to wash during a heavy storm just after planting. On this area every plant was taken by the mildew, while in the remainder of the field not a half dozen died.

Insects also contribute somewhat in conidium dispersal. While diseased corn plants were being examined at night, insects were often seen crawling about on the conidium-bearing leaves and passing indiscriminately from plant to plant. In spite of the fact that many insects are not active at night and that even the nocturnal ones are kept away

to some extent by the moisture on the dew-covered plants, none the less a considerable number were collected on maize during conidium production. These included such diverse forms as several types of leaf-eating larvae, many kinds of ants, and a variety of grasshoppers, leafhoppers, Phryganids, Hemiptera, and Coleoptera. On examination it was found that the appendages and bodies of these insects often had adhering to them large numbers of conidia which germinated readily when placed in dew or on agar. Moreover, in the leaf-eating larvae such as *Plosia* sp., the intestine and even the excrement showed conidia, some of which proved capable of germination when tested. Since the carrying of conidia by insects is apparently of very little importance in comparison to other methods of distribution, no attempt was made to secure further experimental data on frequency of inoculation or distance of spread. It seems clear, however, that occasional infection may occur in this way.

Moist soil with conidia adhering probably serves infrequently as a contributory means toward securing distribution of the downy mildew by other agencies. The damp earth under the low-hanging leaves of small, heavily infected plants occasionally, after still nights, was seen to be whitened with innumerable conidia. Examination showed that these retained their vitality and germinability on the soil until dried by the early sun, while such conidia as were lodged in protecting crannies lived even longer. Moreover, the infection of germinating maize seeds that had not yet emerged from sterile sand was easily accomplished by using bits of this conidium-laden soil as inoculum. The seeds were uncovered, bits of infective soil were placed on their plumules, the sterile sand was replaced, and the pots were protected from further infection. The resulting seedlings were heavily diseased. Undoubtedly seedlings when emerging or just at the surface of the soil are occasionally infected in a somewhat similar fashion in the field. It is probable, also, that such conidium-laden soil is at times splashed about and causes the infection of adjacent maize plants quite as the sporangia of Pythiacystis were found by Smith (*23*) to reach and inoculate low-hanging lemons in bits of spattered earth. Furthermore, it is possible that infective soil could be carried to susceptible young plants by animals, or implements or men. In practice, however, such obstacles as, first, the necessity that the infective soil should be transported before or shortly after dawn, and, second, the necessity that it should reach the susceptible protected parts of the host, would probably prevent this method of distribution and infection from operating in any save rare instances.

DISSEMINATION OF THE PHILIPPINE SCLEROSPORAS

The foregoing discussion of conidium production and dispersal logically leads us to consider the broader subject of the dissemination of the Philippine Sclerosporas. The dissemination of such fungi may be conveniently divided, as Butler (*4*) has shown, into two general types; first, discontinuous over comparatively limited areas by means of a succession of short jumps; second, continuous over large extents of land or water without interruption. Spread of these diseases in the Philippines comprises both types. It is chiefly local and discontinuous, involving short successive steps from field to field and region to region. To a limited extent it is also continuous, involving longer unbroken jumps from island to island over intervening seas. Spread of these diseases to

such distant maize-growing countries as the United States, however, must necessarily be continuous.

The Philippine Sclerosporas can be disseminated in three different stages in their development, as mycelium, as resting spores (oospores with adherent oospore wall), and as conidia. Each of these demands consideration in its relation to the various phases of local and of distant spread in the Philippines or to remote countries.

DISSEMINATION BY MEANS OF MYCELIUM

Mycelium, from its very nature can not be distributed by itself but must be carried with some part of the plant such as cuttings or seed. Infected cuttings do not, of course, enter into the problem as far as maize is concerned, but in the case of sugar cane and of the wild grass, *Saccharum spontaneum*, the Sclerosporas are spread to a limited extent by this means. At Bontoc in the Mountain Province the writer found in January, 1920, that almost all the numerous small patches of cane grown in and around the Igorote villages were heavily infected with an oogonial Sclerospora; and there was strong evidence that the indiscriminate use of infected cuttings had, at least in part, been responsible for its spread. This cane, however, probably because it is a thin, hard-stemmed, purple variety with a rather low yield, had not been distributed widely; and as far as the writer was able to determine the disease is restricted to the one locality. Introduction of this cane into the sugar-producing regions of Calamba, Pampanga, or Negros would be extremely dangerous. Except at Bontoc no instances of distribution of Sclerospora by mycelium in sugar cane were noted in the Philippines. The only other case of Sclerosporal infection of sugar cane seen by the writer was a single plant attacked by the conidial form, *Sclerospora spontanea* (*25*), and so weakened and stunted as to be useless for cuttings. It should be noted, however, that distribution of the Sclerosporas by this means is greatly to be feared, especially by remote countries. It was through the importation of cuttings infected by the mycelium of *Sclerospora sacchari* that this destructive disease was introduced into Formosa from Australia (*15*).[4]

In the case of the wild grass, *Saccharum spontaneum*, also, spread of Sclerospora is accomplished to some extent by infected cuttings. In certain provinces, especially Laguna and Pangasinan, it is a common practice to plant this grass by means of cuttings, as a binder along the newly made retaining walls between rice paddies. In those provinces the oogonial Sclerospora which has previously been mentioned (*25*) is very prevalent on the grass, and as the farmers, ignorant of the true nature of the disease, make no effort to select healthy stems, the mildew is frequently transplanted in the cuttings. As a result, in the newly grown grass borders of these fields the writer commonly encountered extensive infections which in location, age, and other characters gave clear evidence of arising from infected cuttings. Experimental plantings, using diseased stalks, developed similar extensive infections. In the case of plants of this grass parasitized by the conidial *Sclerospora spontanea* the writer also found experimentally that cuttings could carry the disease, but no instances of such transmission of the conidial stage were observed in the field.

[4] Since this was written the writer has learned from Mr. H. A. Lee, of the Philippine Bureau of Science, that *Sclerospora sacchari* has recently been introduced into the Philippines from Formosa in cuttings whose infection remained undetected through inspection in Formosa and Manila and persisted uninjured through precautionary dipping in Bordeaux mixture.

Mycelium-infested seed, in contrast to cuttings, apparently plays no part in the distribution of Philippine maize mildews. Although in maize the writer (*24*) has found Sclerospora mycelium in the seed coats and even invading the endosperm, a series of experimental plantings of such seed in all stages of development, and even with the diseased cob still attached, gave rise to no mildewed plants. Similar plantings of such seed from infected plants of teosinte (*Euchlaena luxurians* Schrad.), teosinte-maize hybrids, and *Saccharum spontaneum* gave like negative results. In the Javan maize Sclerospora, Palm (*16*), after careful histologic study and extensive plantings, found the same to be true. These facts are of interest, as it has long been assumed that seed-borne mycelium is a means by which Sclerospora is distributed. This assumption has been based on investigations carried out on *Sclerospora macrospora* Sacc. in which the oogonial phase alone is known, and on *S. graminicola*, in which the conidial stage is rare and the oogonial preponderates. It should be noted that in the cases recorded infection might well have arisen, not from seed-borne mycelium but from oospores which in both species are produced in vast numbers and were undoubtedly adhering to or present in the coats of the seed used. This explanation would apply to Peglion's observation (*17*) that grain from wheat heads infected with *S. macrospora* and showing mycelium in the outer coats produced, when germinated, abnormal seedlings with mycelium in their tissues. It would also apply to the case described by Massee (*14*) of an outbreak of Sclerospora in England on sugar-cane grown from seed from France, a case that is particularly interesting not only because it presents evidence for seed transmission, but also because the resulting downy mildew was *S. graminicola*, a species as yet unrecorded on cane.

DISSEMINATION BY MEANS OF OOSPORES

When the distribution of the Philippine Sclerosporas by oospores is considered it is impossible to state how much or how little is accomplished by these bodies, since unfortunately the exact role which they play is not at present understood. That they must be functional to some extent in enabling the disease to withstand unfavorable conditions or to spread over distances so great that conidia would be killed in transit seems logical and necessary. Yet, in so far as the writer was able to determine, oospores are never produced on maize in the Philippines. The conidial stage alone occurs on this host, persisting, although somewhat diminished, through unfavorable seasons, spreading disastrously in favorable periods, and causing widespread destruction throughout the islands, all apparently quite independent of any oogonial phase.

So far as their existence on maize is concerned one need consider the Philippine Sclerosporas only in the conidial stage to understand their persistence, their spread, and their destructiveness. But, on the other hand, on other gramineous hosts in the Philippines it is the oogonial stages that are predominately widespread, persistent, and destructive. On *Saccharum spontaneum*, for example, Sclerospora oogonia are extremely abundant, not only in the lowlands where the conidial phase, although rare on this host, is common on maize, but also in the mountainous interior of Luzon where conidia on either host do not occur or are so rare as to escape observation. Similarly, in this same mountainous region the wild grass *Miscanthus japonicus* and the cultivated sugar-cane show abundant infection with oogonia exclusively. Moreover,

this stage alone was found on wild grasses in remotely isolated uplands far from maize, and on islands two or three miles from shore.

The three oogonial Sclerosporas, although morphologically similar, occur on three different hosts and are occasionally found in widely separated localities. The conidial Sclerosporas are morphologically separable as two distinct species (*25*). It is impossible, at present, to understand what relationship, if any, exists between these conidial and oogonial stages. From the evidence at hand it is not apparent whether the conidial *Sclerospora philippinensis*, found on maize, sorghum, and teosinte, and inoculated to *Saccharum spontaneum* and *Miscanthus japonicus*, has as another phase in its life cycle the oogonial stages found on one or more of the three hosts mentioned above, and whether the conidial *Sclerospora spontanea* found on maize, *Saccharum spontaneum*, and sugar-cane, and inoculated to *Miscanthus japonicus* is related to these oogonial forms, or whether all are independent and unrelated.

The solution of this relationship necessitates germinating the oospores and securing conidial infection from inoculation with them. So far the writer's preliminary investigations of these points have resulted unsuccessfully, as have all the attempts reported in the literature by investigators of the genus from its founding by Schroeter (*22*) in 1879 to the present day. To be sure, in 1884, Prilleux (*18*) reported that M. Frechou, paramacien of Nérac, had seen the oospores of *Sclerospora graminicola* germinate by the protrusion of a hypha; but this investigation was not followed far enough to cast any light on the relationship of the two stages. Until this relationship is worked out, the part played by oospores in distributing and maintaining the downy mildews in the Philippines can not be understood.

When considering the Philippine Sclerosporas as a source of danger to the United States it is chiefly with the oogonial stage that we are concerned. Introduction probably would be accomplished by oospores, for as has been seen the fungi could not survive distant transportation except in this stage or as mycelium in sugar-cane or grass cuttings. If established in our country the Sclerosporas would survive the winter principally by means of the oospores which with their adherent oogonia are characteristically thick-walled and resistant. It is in this way that the closely related *Sclerospora graminicola* which thrives in the Tropics is able to live through the year in northern Iowa, Wisconsin, and Michigan. For these reasons the detailed study of the oogonial phases both of the Philippine Sclerosporas and of other species is one of the most pressing needs of future investigation.[5]

DISSEMINATION BY MEANS OF CONIDIA

It is the conidia which play the most important part in disseminating the downy mildews of maize throughout the Philippine Islands. From a knowledge of the details of conidium production and dispersal which

[5] It is of interest to note that since the foregoing was written *Sclerospora macrospora* has been found on wheat in the United States. Wheat plants attacked by this fungus were collected at Jordan, Ky., in April, 1921, by Mr. R. W. Leukel and identified by Dr. A. G. Johnson, who later found the same parasite on specimens of wheat collected in California in 1919. Subsequent investigation by the writer has shown that the Sclerospora is well established in the wheat fields of western Tennessee and Kentucky. This species has never been found in the Americas before, but in Italy, southern France, and other parts of Europe it has been known since 1895 as a disease sporadically destructive to wheat, maize, oats, rice, and various wild grasses. Its occurrence in Tennessee, far from wheat, on *Bromus commutatus* Schard., a grass introduced from Europe within comparatively recent years, possibly indicates that this host brought in the disease with it. In any case, this instance emphasizes the danger of the oogonial stage, the only one known in this species as a means of disseminating Sclerosporas.

have been given above, one is able to understand and adequately to explain the most important phases of the problem presented by these maize mildews in the Philippines. The gradual local spread of the disease in some regions and its sudden scattering over extensive areas in others, the apparent immunity of maize in certain localities and the destruction of the whole crop in others, the sudden appearance of the disease in areas hitherto uninfected and the equally rapid disappearance of long persistent infection elsewhere are all no longer baffling.

It should be noted, however, that the dispersal of conidia does not necessarily imply distribution of the downy mildews. Vast quantities of germinable conidia may be scattered by the breezes; but if they fail to reach susceptible parts of favorable hosts before being killed by drying, no distribution of the Sclerosporas will result. The following points must be borne in mind. To secure the production of large numbers of conidia there must be a heavy and lasting deposition of dew on the infected plants; to effect the dispersal of the conidia so produced there are required night breezes of favorable strength and duration; while to accomplish infection from the quantities of conidia thus scattered there must be available and accessible in the field, within carrying distance, young susceptible maize plants into whose dew-bathed buds and leaf axils the breeze-borne conidia may fall. These conditions are the limiting factors affecting distribution of the downy mildew through the agency of conidia. In the Philippines, climatic conditions and agricultural practices are such that these requirements are very generally fulfilled. As a result dissemination by means of conidia goes on, year in and year out, unhindered.

In achieving discontinuous, step-by-step distribution of the maize mildews, the conidia are preeminently important. At the beginning of each rainy season the downy mildews gradually spread in the newly planted maize from centers where the conidial stage has lived through the dry season on maize planted out of season or maintained under irrigation for forage. Along the edge of the broad Batangas plain, for example, small areas are kept in maize during the dry season at Sto. Tomas, where the proximity of Mount Maquiling insures unseasonable rains, and at Lipa where Mount Malepunyo serves similarly. At both places downy mildew persists on these plantings; and when the rainy season begins the new maize crop, exposed each night to large numbers of wind-borne condidia, becomes rapidly and extensively infected. Also, in the foothills of Mount Banahao, at San Pablo, Lilio, Nagcarlan, and Majayjay numerous densely and successively planted maize plots are maintained through the dry season to supply forage for the tough little ponies that are the chief means of transportation over the steep roads. In these plots the downy mildew is carried along to spread with disastrous effect to the more extensive plantings of the main maize-growing season.

Not only can the progress of the disease be followed in limited areas during early stages in the development of a single maize crop, but in the course of the growing season, which in some regions accommodates two or even three successive crops and occupies two-thirds of the year or more, spread over extensive areas can easily be traced. For example, in the Batangas plain and along the border of the Lake of Bay, where the progress of the maize mildew was followed closely during two years, it was observed to spread from the persistent foci of infection which have been mentioned and extend with ever-increasing severity and expansion

until finally in the last crop maturing in the early part of the on-coming dry season it had penetrated even to relatively remote fields and caused them heavy losses.

Whether the discontinuous spread of the mildews from island to island is due to the agency of conidia could not be settled by observation. Certain evidence, however, seems to indicate that such distribution does occur. The closely aggregated Philippine Islands are idealy situated for the dissemination of the downy mildews by wind-blown conidia. Of the entire 3,000 islands, about 1,000 are of sufficient size and importance to be worthy of agricultural consideration; and these are grouped in an irregular triangle extending about 1,000 miles from north to south. The 11 largest of these extend over more than four-fifths of this distance and comprise but little less than four-fifths of the total area. Yet the distance separating any one of these from the next is not over 20 miles—often much less. Moreover, their arrangement is such that typhoons and prevailing trade winds have a general lengthwise trend over them. Downy mildew of maize is already known from the great Island of Mindanao at the south, from the Visayan Islands in the center, and from Luzon at the far north. Seven of the 11 largest islands are known to harbor the disease; and in all probability investigation will show that it is established throughout the whole archipelago. That this broad distribution has been and is being accomplished, at least in part, by conidia blown from one island to another on night breezes seems very probable to the writer. Night winds, especially those which come in sudden gusts of considerable strength, often have a velocity of 10, 20, or more miles an hour; while the gales which distinguish nocturnal typhoons frequently reach hurricane velocity. Moreover, especially in the Visayan Islands, diseased maize is found in abundance on highlands which unobstructedly overlook the intervening seas. It seems probable that conidia swept by violent gales from such vantage points would take no longer to reach the maize fields of neighboring islands 10 miles distant than conidia borne on almost imperceptible air currents would take to drift from stunted plants to plates exposed 100 feet away.

Butler (*4*), in summing up the general question of wind distribution of fungi, concludes that—

> Several [cases] suggest that aerial spread is limited to a few miles, while other [fungi] are capable of bigger jumps, possibly 50 miles or (in the case of the oak mildew) even more.

The striking experimental evidence of Hesselman (*12*), on the other hand, shows that pollen grains much larger than conidia may be transported 30 and even 50 miles by breezes of moderate strength. It should be remembered, however, that pollen and the spores of most fungi are not so rigidly restricted in time or extent of distribution as are the downy-mildew conidia which can not survive drying and must be produced, scattered, and reach new hosts within relatively few hours of the night. The fact that the writer (*25*) has found two conidial Sclerosporas on maize apparently restricted to different groups of the Philippine Islands must also be considered. The writer inclines to the belief that dissemination of the maize mildews from island to island by means of conidia has taken or is taking place, but until further evidence has been obtained and the geographic distribution of these and other species has been worked out for the whole archipelago, no final decision on this point can be made.

It is clear, however, that the conidial stage of Sclerospora, at least in the Philippine forms, is far more complex in respect to conidium production and dispersal and far more important in disease dissemination than has been realized hitherto.

SUMMARY

In the Philippine Islands the two downy mildews, *Sclerospora philippinensis* and *S. spontanea* are in their conidial phases injurious parasites of maize. Investigation of the two species has cast light of certain hitherto obscure points in: (1) The production and dispersal of conidia, and (2) the relation of these processes to the local and distant dissemination of the diseases.

Production of conidia on infected plants is preceded by the establishing on leaves and sheaths of characteristically discolored areas that in outline and pallor are symptomatical of the disease. Production takes place chiefly and in greatest quantity on such areas which may occupy a relatively large proportion of the plant's surface.

Conidiophores never develop except from the stomata, at night, and when the surface is covered with dew or other moisture. From the mycelium in the underlying tissue, branches push through the stomatal pores, forming crowded groups of lobed and knob-like outgrowths. These elongate, developing successively into mature conidiophores, several of which are formed at each stoma during the course of the night. The conidia apparently are liberated by active ejection from the sterigmata, rather than by passive disjunction as has been assumed; but further investigation is necessary to establish this point. Nocturnal development of conidiophores in the usual amount of dew follows a relatively regular cycle, which, however, may be altered by rain or by drying winds.

Conidia are produced in vast numbers, even a small plant liberating a quantity sufficient to infect the neighborhood. The number produced on a plant during one night chiefly depends on the extent of the productive surface, on the number of stomata in this surface, and on environmental conditions. Approximate estimates of the number produced on representative plants of different ages are given.

Production of conidia on an infected plant is not limited to one night but may be repeated on successive nights when conditions are favorable over a period covering as much as 75 per cent of the total life of the plant. The duration of production in the case of several different varieties of infected maize is given.

Dispersal of conidia takes place necessarily at night. (1) It is accomplished chiefly by wind. Slight air currents are especially important; because, although local, they occur very frequently and at the time of maximum conidiophore production. Stronger breezes, usually near the sea and on broad plains, are effective because they occur often during maximum conidiophore production and cover considerable territory. Violent gales are not conducive to copious and general production of conidia but yet are important, because they rapidly sweep such few conidia as may be formed over great distances. (2) Dispersal of conidia is also accomplished by splashing. Drops of dew or rain falling on the conidiophore-bearing leaves, splash off, laden with conidia, and may reach young maize directly or may be carried to it by wind. (3) Dispersal is

also effected, though to a much less degree, by agents of minor importance, such as surface water, insects, and moist soil laden with conidia.

The genus Sclerospora may be disseminated in three stages of its development—as mycelium, as oospores, and as conidia. (1) Mycelium, in the case of maize itself, plays no part in disseminating the Philippine downy mildews. However, as mycelium in cuttings of other hosts, such as sugar cane and related grasses, these diseases are distributed in the Philippine Islands and thus could be brought to the United States. (2) The oospore stage is not found on maize in the Philippines. Whether the oogonial Sclerosporas occurring on sugar cane and two wild grasses in the islands are related to maize is not known. However, this stage must be responsible for at least some of the local and distant spread of these fungi and may possibly be involved also in the dissemination of the conidial phases on maize. (3) Not only during any one season but from year to year conidia are the most important means of accomplishing both locally and extensively the destructive spread of the downy mildews on maize throughout the Philippine Islands. Spread to the United States could not be accomplished by conidia unless these developed on plants imported alive. If the disease were introduced in any fashion, however, the conidia would be of the greatest importance in rapid dissemination.

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PLATE 1 [1]

A.—Young maize plant (Mangonon White Dent) four days after the first symptomatic paling of the leaves. Conidiophore production which has already taken place on two favorable nights has begun characteristically on the distal portion of the upper leaves (*a*, *a*). The total conidiophore-bearing area at this early period comprises only a few square centimeters but as time goes on will continue to become increasingly extensive as the plant develops. ×½.

B.—A near view of the fifth to eighth leaves of a young maize plant (Chinese Waxy white), showing (at *x*, *x*) the etiolated stripes characteristic of the downy mildew. These markings had only begun to turn pale a little before dawn of the morning on which they were photographed, hence they were still inconspicuous and free from conidiophores. During the succeeding night, however, conidiophores were developed on the pallid areas. Natural size.

C.—Plant of maize (Guam White Flint) which, although only a little over 3 weeks old, is already producing vast quantities of conidiophores each favorable night from relatively extensive and conspicuously pale areas on its upper leaves and sheaths. ×¼.

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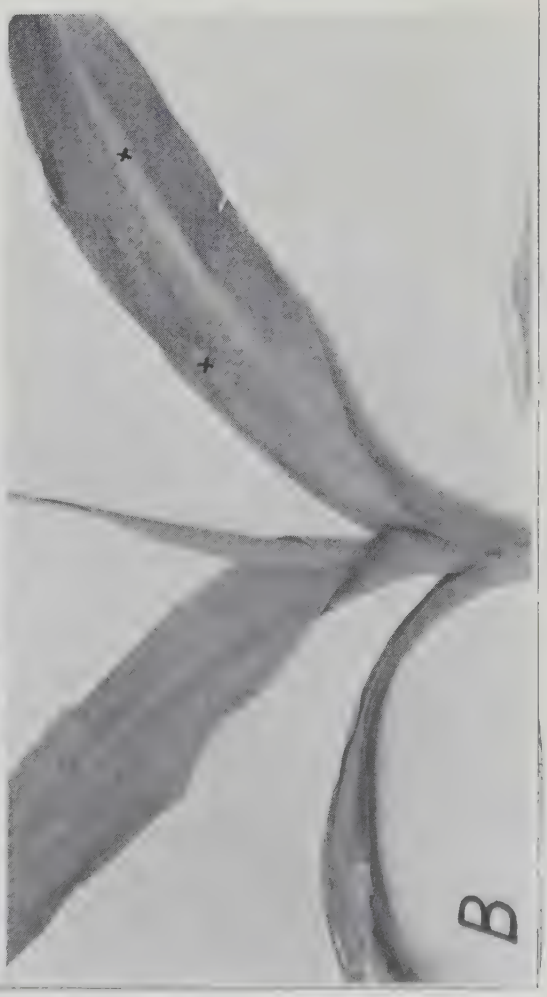
[1] The photographs of Plates 1 to 6 were taken by the author.

G

d
d
A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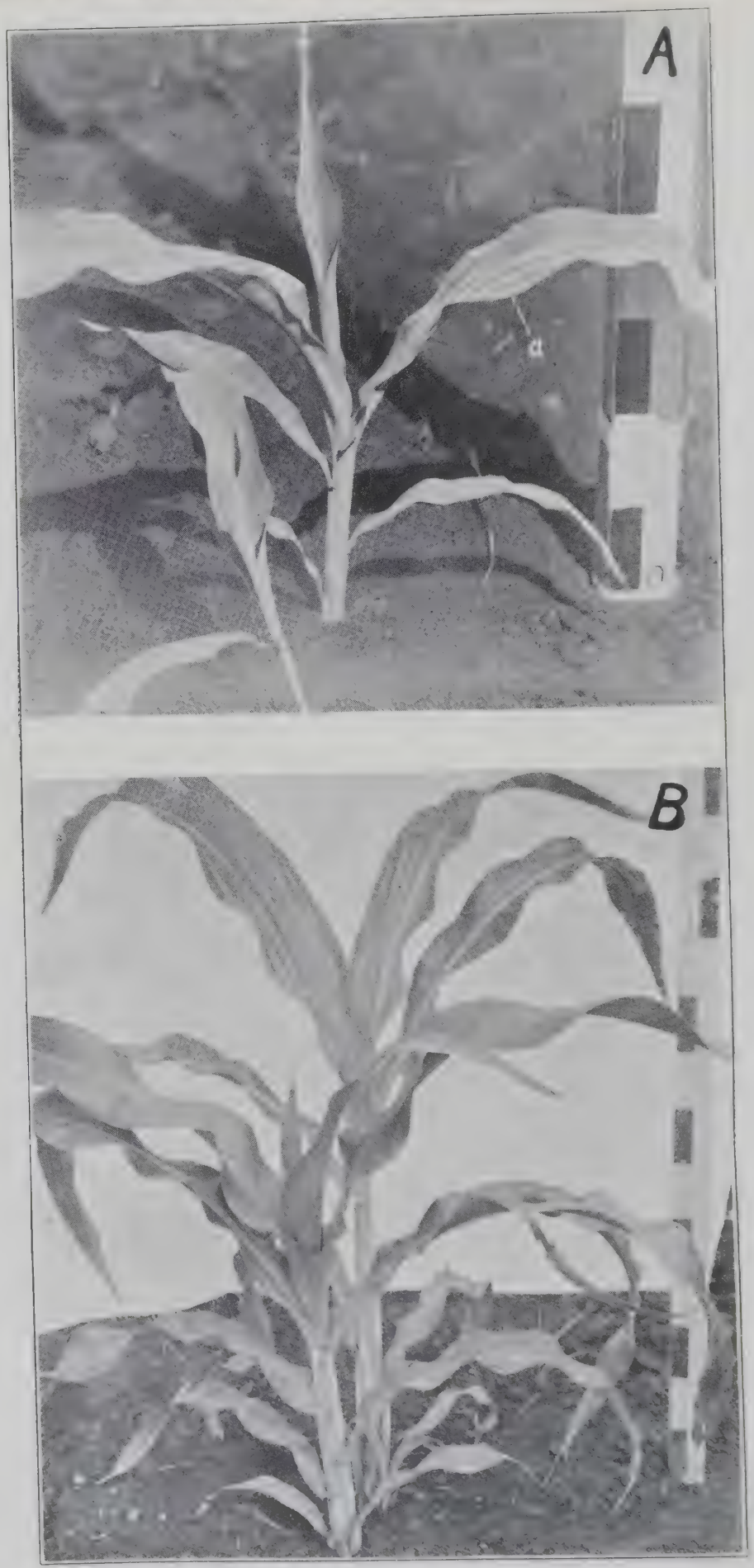

A
B

PLATE 2

A.—Two young maize plants (Guam White Flint) 10 days after emerging; the smaller and nearer one, healthy; the larger one already infected and showing (at *a*) the etiolated conidiophore-bearing areas characteristic of the downy mildew.

B.—The same plants about a month later. The conidiophore-bearing area which was inconspicuous and occupied only a few square centimeters on the leaves of the young plant is now very extensive and conspicuous and supports the production of vast numbers of conidia each favorable night. The meter stick, which is the same as that shown in A, is marked off in 5-cm. divisions.

PLATE 3

A.—Two plants of Manobo Waxy Maize 14 days after emerging; the one at the left healthy, the one at the right already infected with *Sclerospora philippinensis*, and showing etiolated stripes symptomatic of the disease. These stripes are relatively inconspicuous (*a*, *a*) and have a comparatively small area but are supporting abundant conidiophorz production from both their upper and their lower surface. The measure is marked in 5-cm. divisions.

B.—The same plants 16 days later. The etiolated markings which have been developed by the infected plant at the right are now relatively extensive and conspicuous and on them from both surfaces conidiophore production continues to take place in abundance. Measure as in A.

A
a
a
B

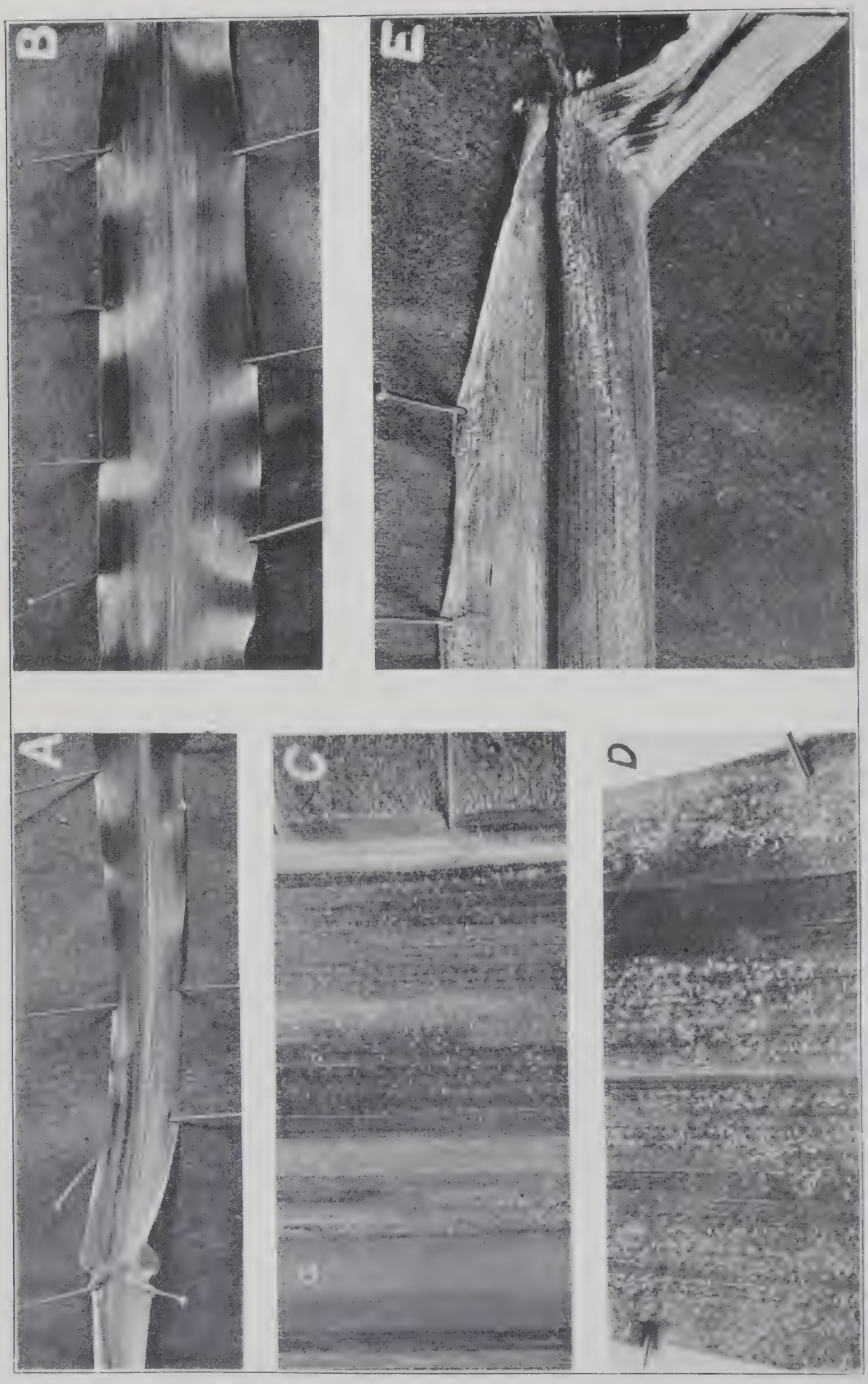

B
E
A
C
D

PLATE 4

A.—Basal portion of the fifth leaf of a young plant (with seven leaves) of Pueblo White maize, showing recently established etiolated markings due to *Sclerospora philippinensis* which extend up from the very pale leaf sheath. Conidiophore production on this lower leaf had not as yet become abundant and conspicuous. Natural size.

B.—Distal portion of the sixth leaf of the same plant. The etiolated areas extend nearly to the tip of this leaf and on them conidiophores have already developed in abundance, especially along the midrib. Natural size.

C.—Portion of a leaf of Moro White maize from a plant heavily infected with *S. philippinensis*. Conidiophore production is most abundant along the pallid stripes but also occurs scatteringly on the less conspicuously discolored areas between them and even on the midrib, *a*. ×2.

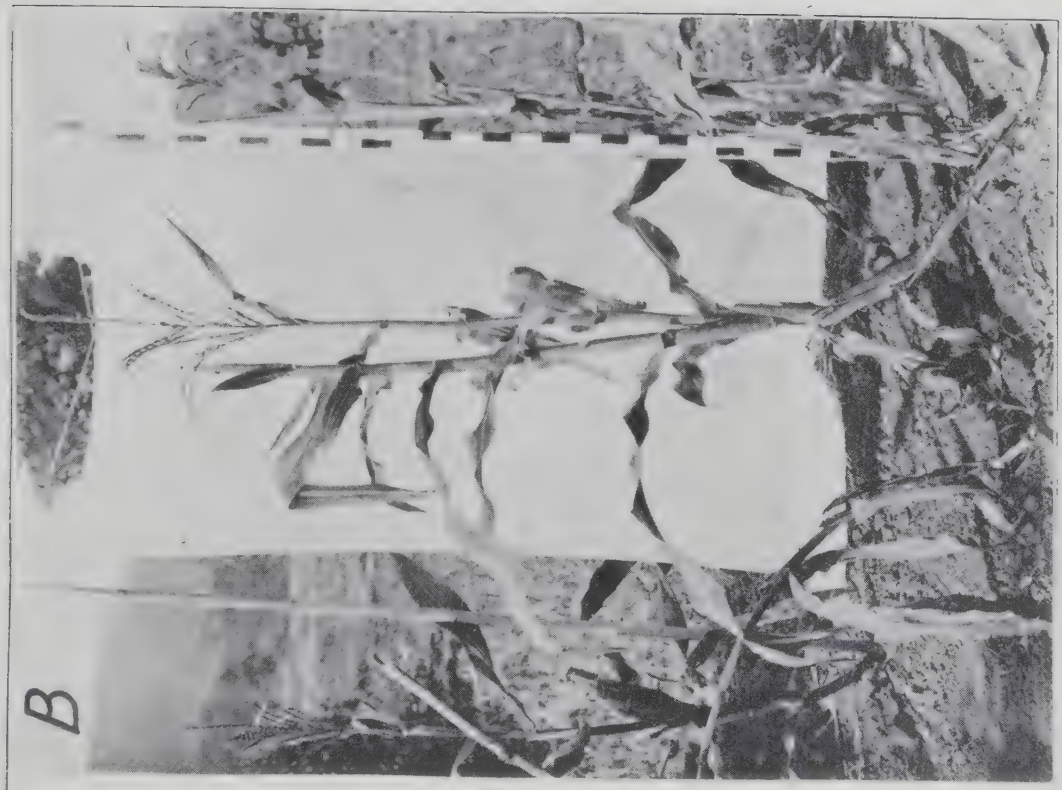
D.—Portion of a leaf of Guam White Flint maize showing abundant production of the conidiophores of *S. philippinensis*. ×2.

E.—Unrolled sheath of the uppermost leaf of a heavily infected plant of Moro White maize, showing conidiophores which were formed in the protection of this overlapping edge during a nocturnal storm that was so severe as to prevent spore formation on more exposed parts. ×1½.

PLATE 5

A.—Deformed, virescent tassel of a plant of Cattete Brazilian Yellow Flint resulting from a severe attack of *Sclerospora philippinensis*. This plant showed the usual heavy production of conidia from its extensive leaf and sheath areas. In addition, from a large proportion of the surface of the abnormally developed bractlike outgrowths in this tassel conidiophore formation took place in abundance.

B.—Plant of Guam White Flint which, as a result of infection by *S. philippinensis*, developed an abnormal ear branch. This branch resulted from the elongation of the shank and the excessive growth of the husks of an ear which at first was apparently normal. Conidiophore production, after it had ceased on the main body of the plant, continued in abundance on the broad, leafy tips and the basal sheath portion of the husks of this branch, prolonging the period of spore production to a total of more than nine weeks. At the extreme left can be seen a healthy plant with a normal ear. The meter stick is marked off in 5-cm. divisions.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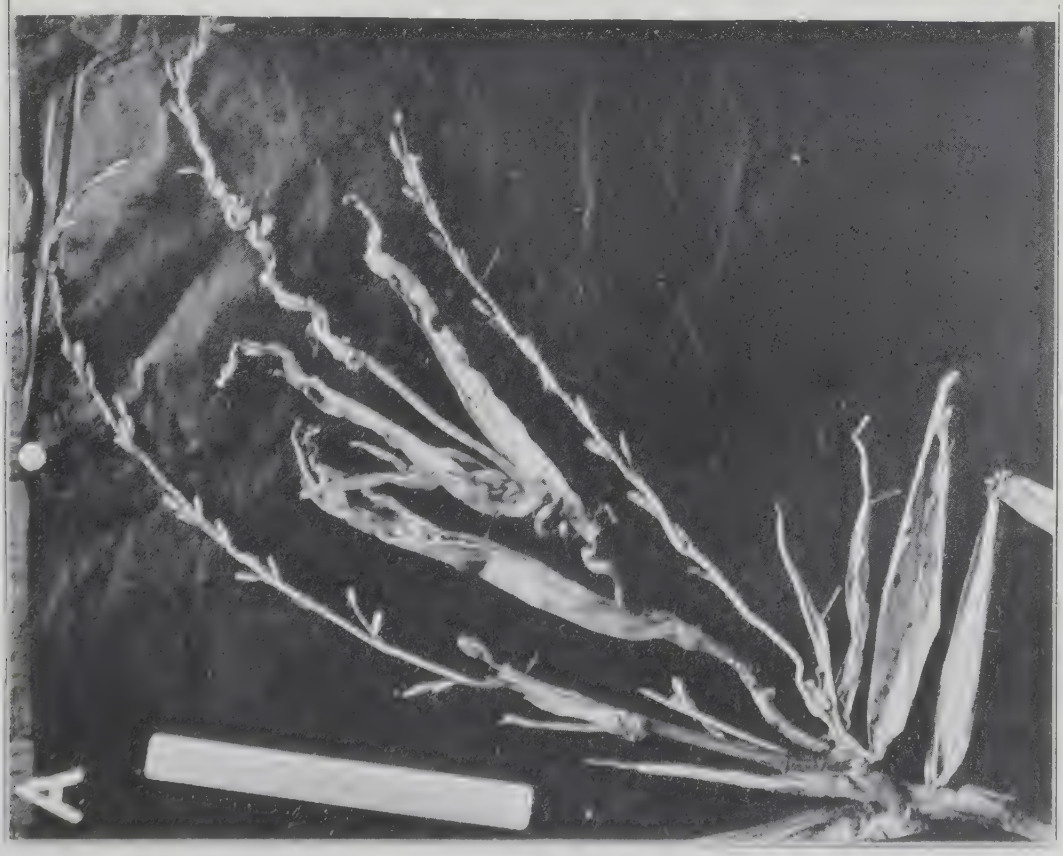
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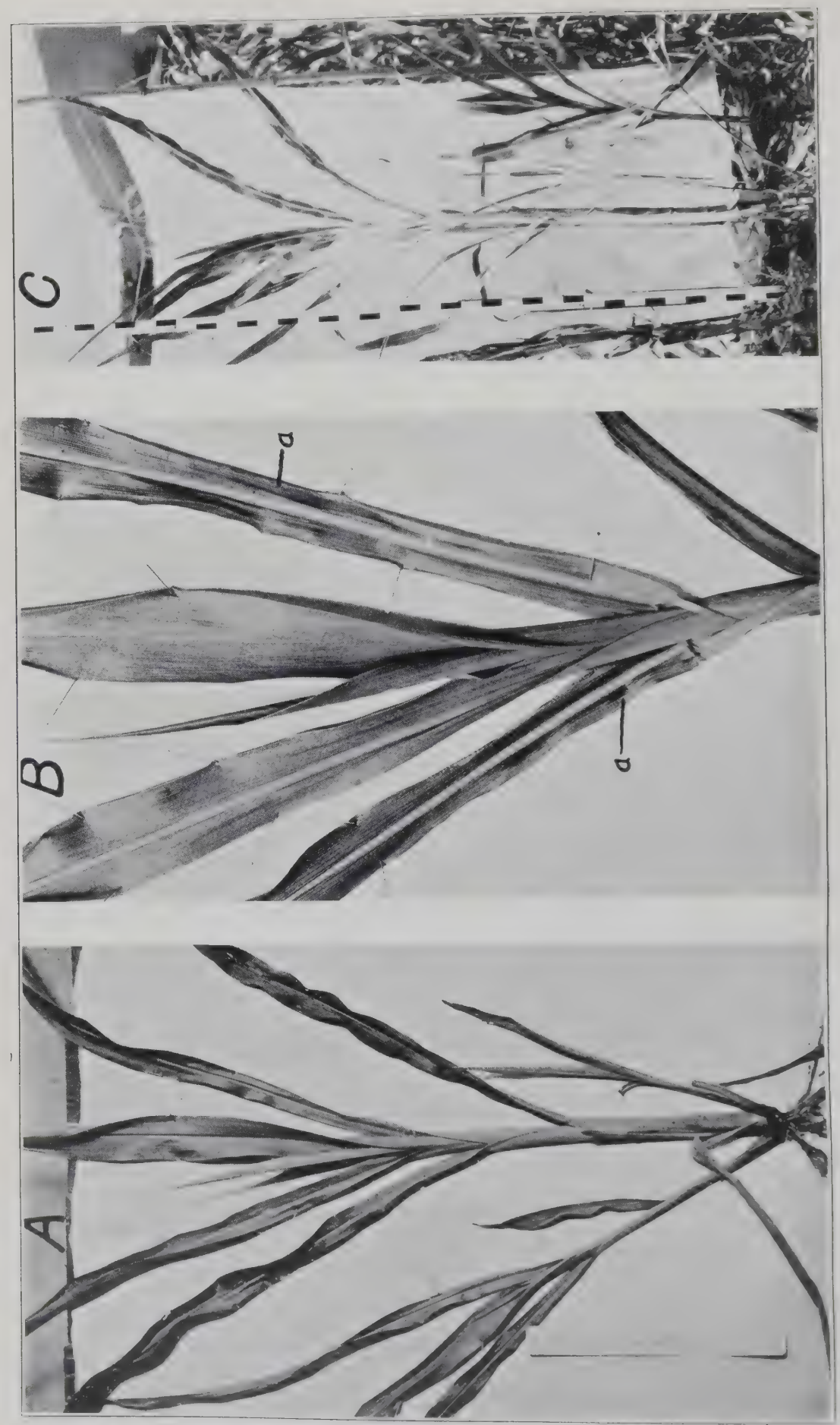
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PLATE 6

A.—A young teosinte plant infected with *Sclerospora philippinensis* and supporting abundant conidiophore production from relatively inconspicuous markings on the leaves, not only of the main shoot but also of the two lateral suckers by which the total spore-producing area in considerably increased.

B.—A closer view of the central shoot of A, showing the pallor of the conidiophore-bearing areas of the leaves and sheaths compared with the isolated stripes of normal green which still persist at *a*, *a*. As can be seen, the pallid portions occupy relatively a large proportion of the total leaf area, and since they support conidiophore production on both surfaces the total amount of production each night is large.

C.—An older teosinte plant growing as an escape in a field of Mexican June maize, from which it has become heavily infected with *S. philippinensis*. Although slender and spindling, the plant has a relatively large conidiophore-bearing surface and liberates great quantities of conidia each favorable night. Each division of the meter stick equals 5 cm.

PLATE 7[1]

A–D.—Stages in the development of the branches which start from the substomatal mycelium, push through the stomatal pore, and by forming lobed outgrowths give rise to the crowded group of knoblike buds that develop into the conidiophores. Maize (Guam White Flint) infected by *Sclerospora spontanea;* exterior view of the epidermis showing stages characteristically found from 10 to 11 p.m. A.—Stomata not yet invaded; its pores closed to a narrow slit. B.—From the substomatal mycelium, part of which can be seen dimly beneath the epidermis at *a* and *b*, many knoblike branch tips are crowding up through the stomatal pore. C.—A later stage in the growth of the knoblike branches, some of which have enlarged and pushed still farther out of the stoma. D.—A still later stage; the knoblike branches have grown into enlarged and lobed outgrowths which will elongate into the club-shaped conidiophore initials. ×700.

E.—A similar stoma seen in longitudinal section at a later stage in the development of the conidiophores. Above the group at *a* is shown the base of a conidiophore that has already lost its conidia and begun to shrink. At *b* is the base of another which is just maturing. Below are several knoblike outgrowths that will develop into conidiophores in their turn. From a longitudinal section of the same material cut at 2.30 a.m. ×700.

F.—A group consisting of outgrowths and their maturing conidiophores. Similar to that shown in E but dissected out from the surrounding tissue. The maturing conidiophore whose base is shown at *a* is figured in full in Plate 8, Q. 2.30 a.m. ×700.

G.—Portion of a cross section of the same material, showing in the substomatal chamber vigorous mycelial branches from which has arisen a group of knoblike outgrowths. At *a* is the basal extremity of a conidiophore already matured. 2.30 a.m. ×700.

H.—Portion of a cross section of *Saccharum sponatneum* leaf heavily infected with *Sclerospora spontanea.* From the stoma, which, in this grass, is deeply depressed and protected by ridges, has pushed out a small group of outgrowths one of which has begun to elongate into a conidiophore. Their relationship to the vigorous mycelial branches in the substomatal chamber can be traced clearly. 2 a.m. ×700.

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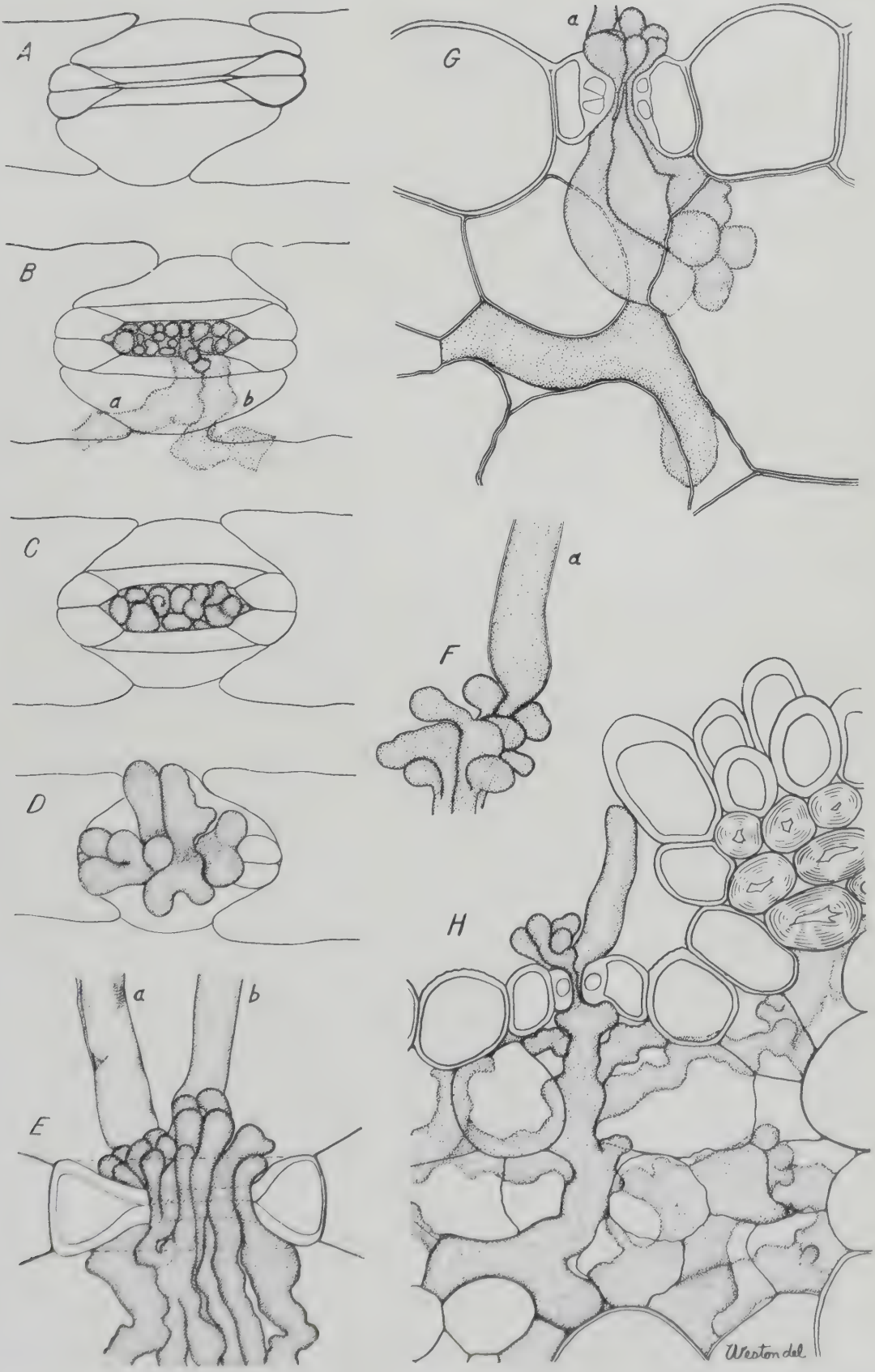
[1] The drawings were made with the aid of a camera lucida and are from living material hand sectioned at night in water, the sections being immediately killed and fixed in osmic acid, stained, and mounted.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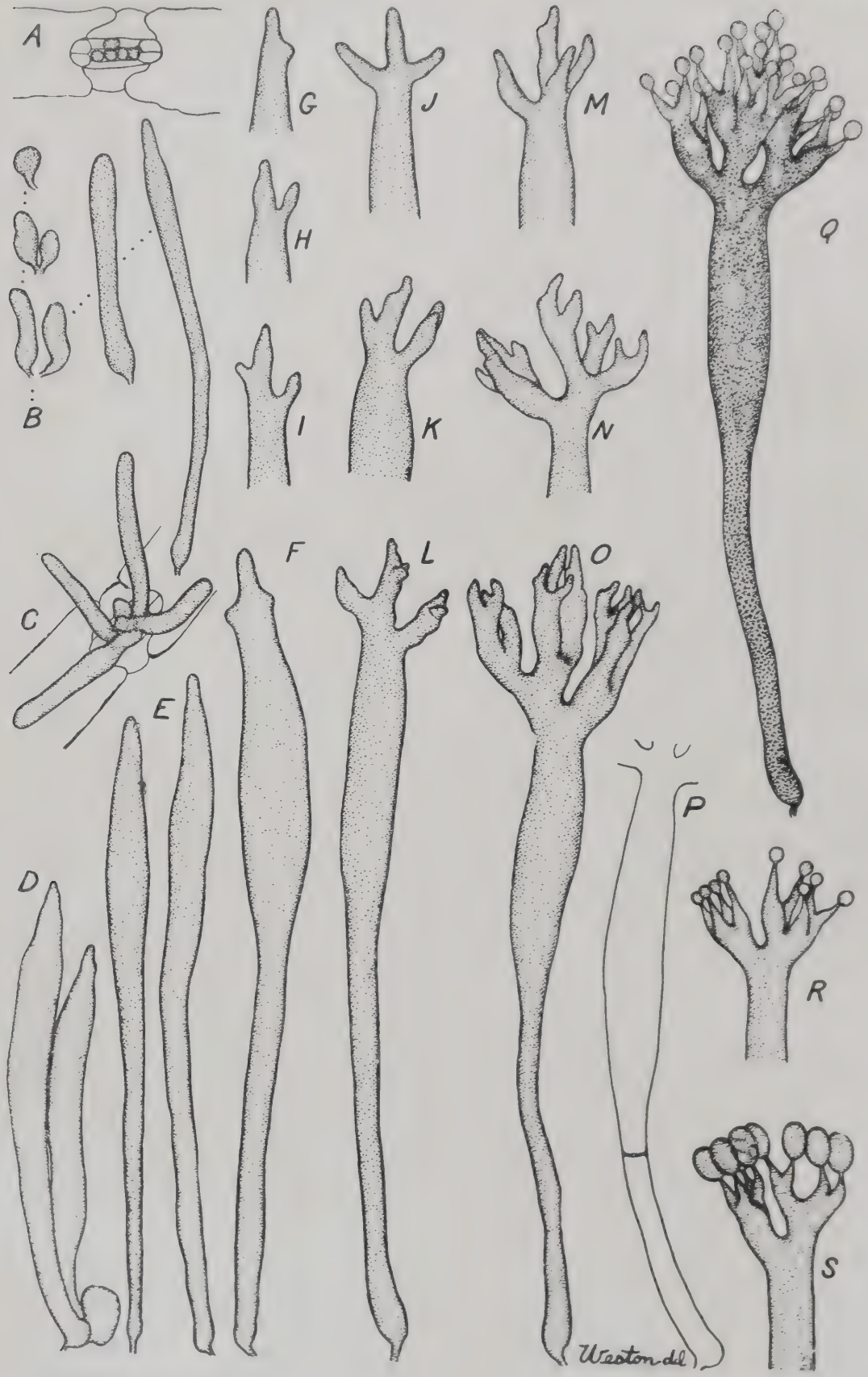
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PLATE 8[1]

A.—External view of stoma ofmaize, showing knoblike outgrowths from the internal mycelium throughout the distended pore. 10 p. m. ×375.

B.—Early stages in the development of conidiophores, showing gradual elongation of the small knoblike outgrowths into club-shaped initials. 10 to 11 p. m. ×375.

C.—External view of the stoma of maize (Barbados Yellow Flint) infected with *Sclerospore philippinensis*. Arising from the stomatal pore are four partly developed initials while a small knoblike outgrowth is pushing up between their bases. 11 p. m. ×375.

D.—Club-shaped conidiophore initials of *Sclerospora philippinensis*. Note the short, stocky habit and the indications that the basal cell will be short and stout. 11 to 12 p. m. ×375.

E.—Conidiophore initials of *Sclerospora spontanea* at approximately the same stage of their development. In comparison to those of the other species (D) they are longer and more slender in habit, especially in the lower portion that will be cut off as the basal cell. 11 to 12 p. m. ×375.

F.—Conidiophore initials at a later stage than those in E, showing the buds of the primary branches beginning to grow out. 11 to 12 p. m. ×375.

G–O.—Conidiophores showing later stages in the development of the branch system. 11 p. m. to 12.30 a. m. ×375.

P.—Conidiophore of *Sclerospora philippinensis* at approximately the same stage of development as the *Sclerospora spontanea* conidiophore (O). Note that the basal cell is shorter and is cut off by a cross wall at a relatively earlier age. 12.30 a. m. ×375.

Q.—Conidiophore showing conidia budding out from the sterigmata tips. Attempt has been made to show the contrast between the finely granular content of the sterigmata and the young conidia to the dense, coarse protoplasm of the main axes and branches. This conidiophore arose from the group of young knoblike outgrowths shown in Plate 7, F. 2.30 a. m. ×375.

R, S.—Later stages in the development of the conidia. 2 to 3 a. m. ×375.

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[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained, and mounted. The figures, with the exception of *C*, *D*, and *P*, show successive stages in the development of Sclerospora on maize (Guam White Dent) which had been inoculated with *Sclerospora spontanea* taken from *Saccharum spontaneum*. Figures *C*, *D*, and *P* are of *Sclerospora philippinensis* for comparison.

PLATE 9[1]

A–K.—Successive stages in the development of the conidia of *Sclerospora spontanea* from the tips of the sterigmata. Material found on maize (Guam White Flint). 2 to 3 a. m. ×1000. A–H.—The conidium enlarges gradually as more and more protoplasm flows into it from the sterigma. I.—At the junction of the sterigma tip and base of the conidium a hyaline zone is differentiated. J.—In this zone a wall is formed across the papilla of attachment of the conidium and likewise one across the tip of the sterigma. K.—Both walls are bulged outward increasingly by the turgidity of the contents.

L.—Conidium of *S. spontanea* which apparently has been broken off prematurely from the sterigma. Note the basal papilla. 2.30 a. m. ×1,000.

M.—Sterigma from which the conidium has been broken off prematurely. Most of the sterigmata on this conidiophore still retained their conidia in situ in the condition shown at J. Compare with P and Q. 2.30 a. m. ×1,000.

N.—Conidium of *S. spontanea* which, even after germinating, still indicates by the character of its papilla that it was broken off. 2.30 a. m. ×1,000.

O.—Conidium of *S. spontanea* that in the normal fashion has been thrown off from the sterigma. Note the bulged out papilla of attachment. 2.30 a. m. ×1,000.

P.—Sterigmata of *S. spontanea* from which the conidia recently have been thrown off. Note the characteristic shape of the bulged-out apices. The content has begun to show signs of disintegration. 2.30 a. m. ×1,000.

Q.—Sterigmata of *S. spontanea*. The characteristic shape of the tip is unchanged even in sterigmata of widely different sizes and shapes. 2.30 a. m. ×1,000.

R.—Sterigmata of *S. spontanea* long after conidium discharge. The content is disintegrating and the walls have begun to collapse, a condition characteristic of conidiophores like that shown in Plate 10, D. 4 a. m. ×1,000.

S.—Shriveled sterigmata with disintegrated content, such as are found on the dried-up conidiophore of *S. spontanea* shown in Plate 10, E. 3 p. m. ×1,000.

T.—Conidium of *S. philippinensis* that in normal fashion has been thrown off from the sterigmata. Note the bulged basal papilla. Material taken from maize (Barbados Yellow Flint). 2.30 a. m. ×1,000.

U, V.—Sterigmata of *S. philippinensis* from which the conidia have been thrown off. Note that the characteristic shape of the bulged tip is unaltered in sterigmata of different sizes. The same material as T. 2.30 a. m. ×1,000.

W.—Sterigmata of *S. philippinensis* from one of which the conidium has already been thrown off, while on the other the mature conidium is still retained in place. The same material as T. 2.30 a. m. ×1,000.

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[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained and mounted.

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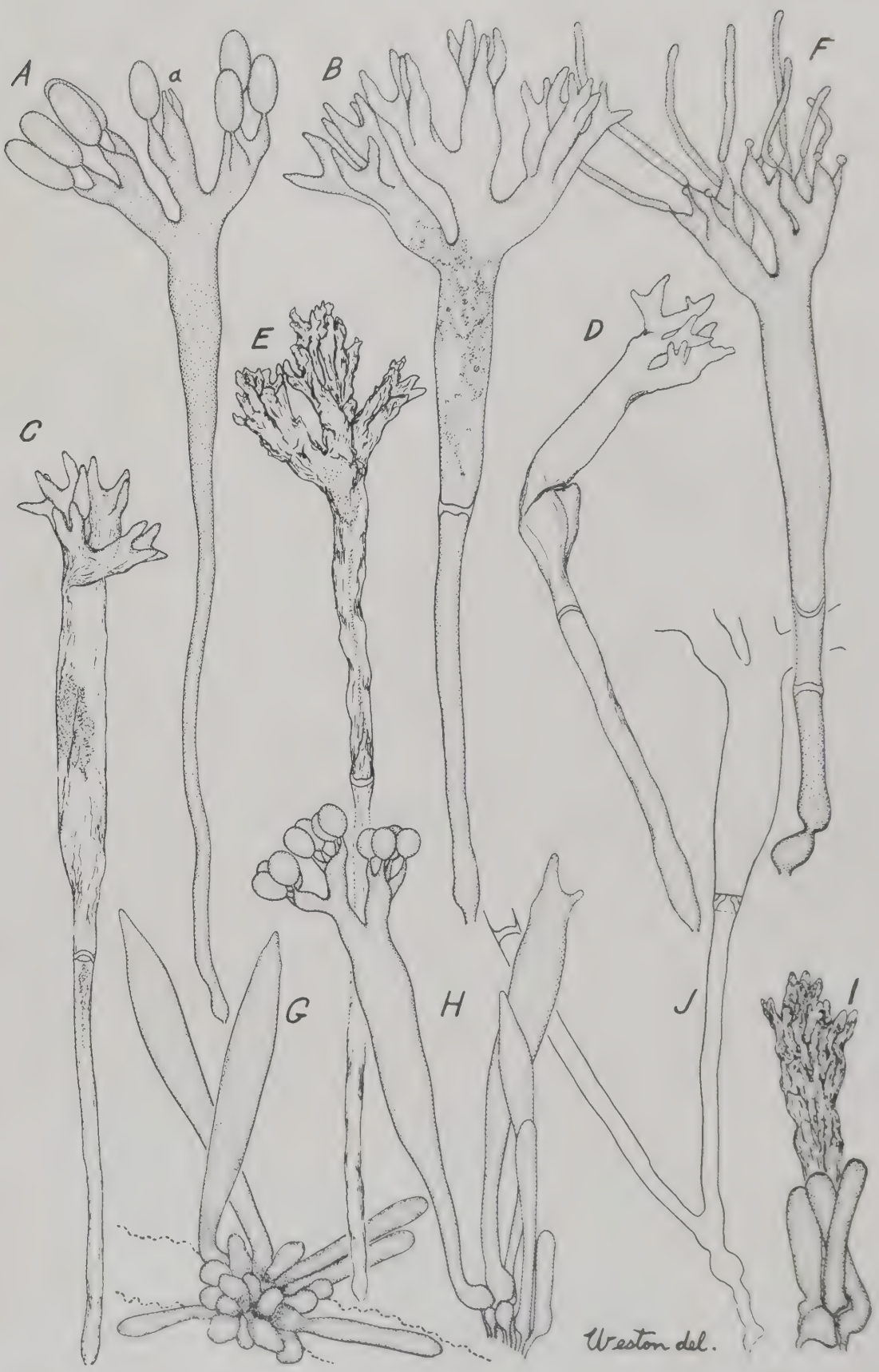
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PLATE 10 [1]

A.—Conidiophore and maturing conidia of *Sclerospora spontanea* on maize (Guam White Flint). Note the truncate apices of the sterigmata at *a* from which the conidia have been broken off prematurely. 2 a. m. ×375.

B.—Similar conidiophore from which the conidia recently have been thrown off. Note that the apices of the sterigmata from which the conidia have been thrown are bulged out. Now that it has functioned the content of the conidiophore begins to disintegrate. 3 a. m. ×375.

C.—Similar conidiophore at a later stage; the sterigmata are little altered but the main axis is losing its turgidity and becoming shrunken. 4 a. m. ×375.

D.—Similar conidiophore still later. The sterigmata and branches have shrunk together and the main axis is shriveling. 5 a. m. ×375.

E.—Similar conidiophore after being dried on the leaf during the day to a wrinkled mummy that is hardly recognizable as the remains of the vigorous structure of the preceding night. 3 p. m. ×375.

F.—Conidiophore of *S. philippinensis* on maize (Barbados Yellow Flint) which shows an unusual formation of hyphae similar to germ tubes from sterigmata that had just begun to bud out conidia. Apparently the conidiophore, having inherent in its protoplasm potentialities for hyphal germination, has for some reason behaved as a multiple conidium. 2.30 a. m. ×375.

G.—Group of *S. philippinensis* conidiophores views obliquely from above, comprising a relatively large number of young stages and completely covering the stoma; from maize (Barbados Yellow Flint) at 2 a. m. ×375.

H.—Similar group of conidiophores, one maturing conidia, and the others in various earlier stages of development, teased out from the surrounding tissue of a leaf section of maize (Barbados Yellow Flint) at 2 a. m. ×375.

I.—Similar group of conidiophores, comprising three club-shaped conidiophore initials and one shriveled vestige of a conidiophore produced probably on some previous night, teased out from the stoma of maize (Barbados Yellow Flint) at 2.30 a. m. ×375.

J.—Unusual basal cell of forked structure serving as the base for two *S. spontanea* conidiophores from maize (Guam White Flint). 2.30 a. m. ×375.

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[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained, and mounted.

MECHANICS OF INOCULATION WITH SUGAR-CANE MOSAIC BY INSECT VECTORS[1]

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INTRODUCTION

It has been demonstrated with practical certainty that the only method for natural spread of sugar-cane or grass mosaic is through the agency of insects, acting as simple vectors or possibly as intermediate hosts.

It was established by the writer (*1*) [2] that *Aphis maidis* is able to transmit this disease. A considerable amount of confirmation of this result has now accumulated and notwithstanding some early skepticism it appears to be generally accepted. The mere fact of its ability to function as a vector, however, has been regarded by several investigators as of no great practical significance on account of the supposed scarcity of the insect on sugar cane. It was even suggested that *A. maidis* never occurred on sugar cane and that when colonized on cane plants the insects would die. On the contrary, other investigators have suggested that *A. maidis* is responsible for much of the natural transmission of mosaic in commercial plantings of sugar cane. In view of these divergent opinions, a review of experimental work on this subject is desirable.

REVIEW OF EVIDENCE ON INSECT TRANSMISSION

Taking up the published work in chronological order, Brandes (*1*) announced that *Aphis maidis* is able to transmit the disease to healthy plants after a previous feeding on diseased plants. It was stated in this paper that all species of insects used in the experiments were known to feed on sugar cane and that *A. maidis* in particular had been reported on sugar cane from practically all sugar-cane countries. Thus it was assumed that a certain amount of natural transmission in the field is traceable to this insect. The belief was expressed, however, that natural transmission is not restricted to *A. maidis*.

Smythe (*8*, *9*) carried on a large number of experiments on insect transmission of mosaic at the Insular Experiment Station at Rio Piedras, Porto Rico. He reports successful transmission with a variety of insects. His results, however, are not convincing. The small number of experiments which he reports as successful are questionable, since in some cases his test plants were transplanted to an open field and left for a period greater than the minimum incubation period for mosaic before showing symptoms.

Tower (*10*), also working in Porto Rico, got only negative results from experiments in which he used sucking and chewing insects.

[1] Accepted for publication Nov. 17, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 283.

Ledeboer (6), in Java, announced that he had obtained results which indicated that *Aphis sacchari* as well as *A. maidis* could transmit the disease. In a later publication (7) he states that while his experiments with *A. maidis* had confirmed the work of Brandes (1), his preliminary experiments with *A. sacchari* were not now considered conclusive. He made the observation in this paper that *A. maidis* was noticed to be very abundant on *Panicum colonum* and *Paspalum sanguinale* in the sugar-cane fields and that the winged forms occasionally flew from these grasses to the sugar cane. He states further that these grasses are subject to the cane mosaic.

Bruner (2) published the results of careful experiments with a great variety of cane insects conducted at Santiago de las Vegas, Cuba. *Aphis maidis* alone was able to serve as a vector of mosaic. He states, however, that this insect does not attack sugar cane under field conditions and therefore can not be held responsible for dissemination of the disease in nature.

Wolcott (11), who studied insects in relation to this disease in Porto Rico, states in reference to *Aphis maidis:*

> The Corn aphis has not been found on cane in Porto Rico. It can not in any way be considered responsible for the transmission of mosaic disease of sugar cane under field conditions in Porto Rico.

In another publication (12) he says:

> . . . Mr. Smythe was unable to keep it [*Aphis maidis*] alive on sugar cane in Porto Rico. The experimental transmission by an insect which will live on sugar cane only under abnormal conditions [referring to the experiments of Brandes] proves nothing as to the insect responsible for transmission in the field, except to indicate the place on the cane plant where the insect must feed to cause the disease.

Kunkel (5), in Hawaii, performed careful experiments in which he confirmed the ability of *Aphis maidis* to transmit the disease, but was unable to demonstrate this ability on the part of *A. sacchari*. He also proves convincingly that the corn leafhopper (*Peregrinus maidis*) is able to transmit the disease from corn to corn but, strangely enough, not to sugar cane. Kunkel makes the important observation that a rapid spread of mosaic in sugar cane followed the weeding of a field. In the case cited, the field was infested with goose grass (*Eleusine indica*), some of which had the mosaic. *A. maidis* was abundant on the goose grass previous to weeding, and probably migrated to the cane at that time.

Chardon and Veve (4) conducted experiments in Porto Rico in which mosaic and healthy sugar-cane plants in a field were covered with cheese cloth, and *A. maidis* was introduced into the cage thus formed. The insects colonized on various grasses, including *Eleusine indica*, *Echinochloa colona*, and *Panicum barbinode*, which were growing as weeds in the cage. The weeds were then cut down and the aphids were observed to migrate to the cane. About 65 per cent of the healthy plants in this cage became mosaic during a period of 66 days, the first case appearing 14 days after the migration. In a second similar cage, into which no specimens of *A. maidis* were introduced, no cases appeared among the healthy plants. A somewhat similar experiment with a different species of plant louse (*Cardinaia* sp.) which occurs on the sedge, *Cyperus rotundus*, was reported in the same paper. Four out of six plants in the experiment became mosaic, but since no control plants appear to have been used it needs repetition under better controlled conditions.

A study of these results seems to afford abundant confirmation of the fundamental fact involved—namely, that *A. maidis* acts as a vector of sugar cane or, more properly, grass mosaic. Experiments with positive and convincing results have been performed in the United States, Java, Cuba, Hawaii, and Porto Rico. The fact also appears to be established that while *A. maidis* prefers other grasses to cane as a source of food, it frequently migrates to the cane in large numbers. Weeding of a cane field is evidently a prime factor in bringing about this result, but the present writer has observed infestation of sugar cane by *A. maidis* in Florida in fields which are never weeded. The conclusions of investigators who disclaim that any practical importance attaches to this insect as a vector of mosaic is based on negative evidence, and therefore can not be held to controvert these facts in any way. Evidence has been adduced indicating that two other insects, *Peregrinus maidis* and *Carolinaia* sp., may transmit the disease.

MECHANICS OF INOCULATION

In an endeavor to gain some insight into the method by which insect vectors inoculate plants with sugar-cane mosaic, a number of individuals of several species of sucking insects were killed *in situ*, embedded in paraffin, sectioned and stained. The insects used were *Aphis maidis*, the known vector of mosaic; *Peregrinus maidis*, the corn leafhopper reported by Kunkel (5) to transmit mosaic from corn to corn; and *Draeculacephala mollipes*, the large leafhopper which is so abundant on cane in this country, but which has failed to transmit the disease in repeated experiments. The latter insect was examined for comparative purposes, to determine if possible whether structural or functional feeding phenomena might inhibit its ability to transmit the disease.

It was found that various methods had to be devised for killing the various insects in the feeding posture and holding them so during all the steps of fixing, dehydrating, infiltrating, and embedding. With *Aphis maidis* the process was found to be comparatively simple. A young leaf of corn with a quiet feeding colony was clipped from the plant and thrust into nearly boiling Carnoy's fluid. The insects were apparently killed almost instantly, before they could withdraw their setae, and it was only necessary to transfer the pieces of leaf with attached insects through the succeeding reagents with utmost care in order to prevent agitation of the liquids and consequent dislodging of the insects. In the case of *Peregrinus maidis*, a colony of adults and nymphs on a corn leaf was cooled for 20 minutes at a temperature of 21° F., and when the insects were rendered inactive by cold the leaf was plunged into the hot Carnoy's fluid. A large percentage of these insects floated off but some remained attached. Specimens of *Draeculacephala mollipes*, comparatively large and heavy as well as active leafhoppers, were handled individually in small, moist chambers to the inside wall of which a piece of corn leaf had been pressed and held in a vertical position by means of gum arabic. The insect was gradually rendered inactive by cold while feeding, then suddenly pressed against the stationary leaf with the index finger and at the same instant covered with Carnoy's fluid as hot as could be borne by the finger. Since these insects possess very large and thick feeding setae, as compared with aphids, and therefore do not thrust the setae into the tissues to a great depth compared to the diameter of setae and weight of the insect, as in the case of the

aphids, it is readily seen that their position on the leaf after being killed is relatively insecure. They must be fastened in some way, and this was accomplished by using small drops of collodion as a cement to hold the feet in position. Since this material is soluble in absolute alcohol it was replaced with cellulose acetate when the insects were transferred to this reagent. The cellulose acetate, being soluble in chloroform, could not be used during fixing in Carnoy's fluid following killing. Sections 10μ and 12μ in thickness were obtained and were stained with safranin and gentian violet.

It was demonstrated that with *Aphis maidis* the beak is usually placed on the cuticle covering a stomate guard cell at the point where the cuticle is thinnest (Pl. 1, A) and the setae thrust into the latter by pressure. During this process a copious secretion is excreted at the end of the setae from the salivary glands. The secretion is colored brilliant red by the safranin. This secretion continues to pour from the tip of the setae as the latter passes into the deeper tissues, and forms the sheath described for other aphids by Büsgen (*3*) in his work on honey dew. Typically, the setae of *A. maidis* pass through the sub-stomatal cavity, then through the mesophyl cells either intercellularly or intracellulary, continuing between two cells of the starch sheath and finally into the phloem of the vascular bundle. (Pl. 1, B.) If the ligmified secondary thickening of the xylem elements are encountered, they get no farther in this direction. (Pl. 1, C, D.) In one section (Pl. 2, C) the preference of this insect for phloem is well illustrated, since an exploratory puncture leading to the xylem elements of the bundle is indicated by the empty sheath, while the setae which were partly withdrawn and again pushed forward are shown leading around the xylem and turning in the direction of the phloem. During the whole process the copious secretion from the insect pours into the practically uninjured and rapidly growing tissues of the leaf. The fact that it leads to the phloem cells, rich in substances of known nutritive value for microorganisms, seems especially significant. In all three sections of the series illustrated in Plate 2 the unusual amount of salivary secretion is clearly shown. It appears to the writer that this secretion is unquestionably the medium by which the infective principle of mosaic is carried into the plant. A more perfect mechanism for inoculation could scarcely be devised. There is no apparent wound reaction on the part of the plant; none could be seen in the phloem at any rate, and certainly none is apparent from the outside. Reaction to the virus itself does not cause great structural changes, as is well known. This phenomenon meets the requirements for successful inoculation in this disease in a peculiarly fitting way. It has been found that a definite, measurable quantity of "virus" is necessary. This would eliminate from consideration as an explanation the carrying into the plant of the scanty amount of virulent material adhering to the minute mouth parts. It is necessary to introduce the virus into rapidly growing tissues in the interior of the plant. Even in the much more easily communicated tobacco mosaic, a wound such as the crushing of trichomes seems necessary. Thus the anal secretion or honey dew falling on the normally unwounded surface leaves much to be desired as an explanation of the infection process. Aside, then, from the perfect adaptation of the setae to the requirements for successful inoculation, the phenomenon herein illustrated is the most likely explanation of the transfer of mosaic.

Sections of the leafhoppers, *Peregrinus maidis* and *Draeculacephala mollipes*, do not indicate that the phloem is especially sought during feeding. The evidence points more to the tracheae as the object of attack, but in the case of *D. mollipes* it is impossible to determine this point, since the relatively enormous setae are as great in diameter as the entire vascular bundles found in the young leaves where they prefer to feed. Nothing definite can be stated in regard to the feeding phenomena of *D. mollipes* which would exclude it as a possible carrier. All cells in the path of the setae, however, are crushed or engulfed in their entirety. There is some evidence of salivary excretion even in this large leafhopper, since a few traces of the brilliant red can be seen along the course of the puncture, but no definite sheath could be demonstrated. It would appear quite possible for this insect to penetrate to the vascular bundles by mechanical pressure alone instead of by the digesting or dissolving action of saliva which is quite necessary to account for the penetration of setae in the case of *Aphis maidis*.

These studies are being continued with virulent as well as nonvirulent aphids in parallel series in the hope that more light may be thrown on this problem and also on the etiology of the disease.

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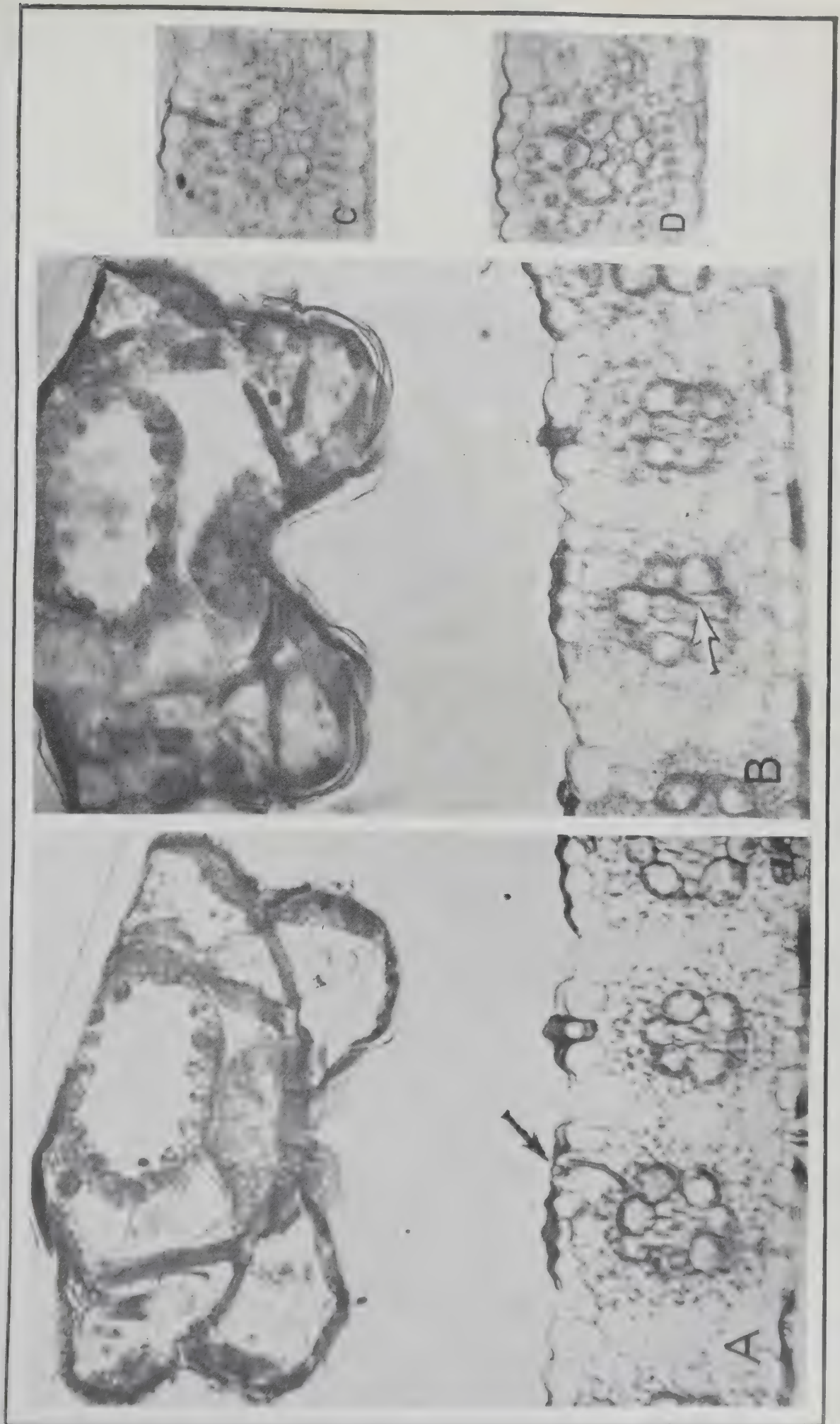
PLATE 1

A.—Setae of *Aphis maidis* penetrating a young corn leaf. The black arrow points to the thin spot in the cuticular covering of a stomate guard cell where the setae entered.

B.—Continuation of the same setae shown in A. The white arrow points to the tip of setae lying in the phloem. This is the typical condition.

C.—Setae entering between epidermal cells.

D.—Same setae shown in C. The setae bundle has encountered a xylem element.


C
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B
A

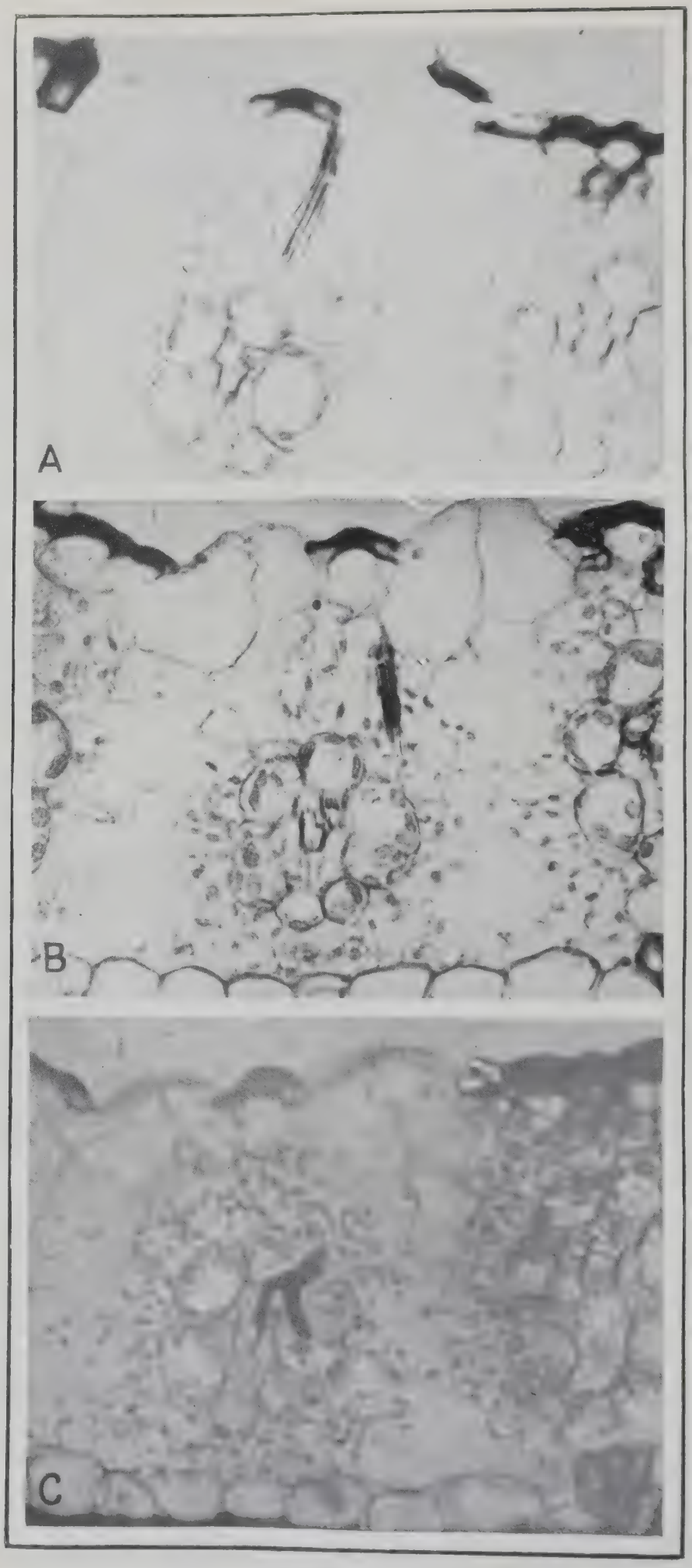
A
B
C

PLATE 2

A.—Setae of *Aphis maidis* penetrating the epidermis of young corn leaf.
B.—Same serae shown in A. This illustrates the copious salivary secretion.
C.—Same setae shown in A and B. An exploratory puncture to the xylem is indicated by the saliva sheath at the left. The setae bundle was withdrawn part way and again advanced toward the phloem.

RELATION OF POTATO SKINSPOT TO POWDERY SCAB[1]

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INTRODUCTION

The skinspot of the Irish potato tuber was first discovered, described, and named in England. It is said to be of considerable importance on seed potatoes, as the eyes may be injured and even killed by the causal organism, and this obviously would result in uneven stands and reduced yields. The same disease has been reported from Canada, and an apparently identical trouble has been described in Germany. It has also been noted on some shipments of Danish potatoes consigned to New York. Its presence in the United States was unknown until some wart-resistant varieties were imported from England and planted in the wart region of Pennsylvania. The progeny of these varieties showed a small percentage of skinspot. Further influx of infected material to this country is very likely to occur as long as the importation of foreign potatoes continues. It is therefore important to ascertain the nature of this disease and the possibilities of its spread in the United States.

SYMPTOMS AND ALLEGED CAUSES OF SKINSPOT

Owen (7) [2] stated very correctly that the external characters of skinspot are so distinctive that there is not much likelihood of confusing it with other spots. The appearance of the disease may be briefly described as follows: Round, raised, closed pustules frequently with a depressed border, single or in aggregates of varied configuration (Pl. 1, 2); dark brown or bluish brown outside and olive brown to brown inside. If the skin of the tuber is thick and rough, whether naturally or through the action of fungi or other causes, the spots are usually larger in diameter. Occasionally the pustules are lenticular or irregular in shape. Often the potato skin is attacked by *Spondylocladium atrovirens*, which gives it a glistening silvery appearance; but this is not characteristic of skinspot. In some cases the spots are not convex but are flat and only slightly raised and of considerably larger dimensions (Pl. 1, B). The disease is so typical and peculiar that no difficulty has been experienced by workers in recognizing it either on specimens or on photographs. The nature of the trouble, on the other hand, is less obvious, and the opinions of various investigators on this point are greatly at variance.

Carruthers (*1*) was the first to record what has been assumed to be, and presumably is, this disease. He found an abundance of fine mycelium in dead cells of the pustules but did not identify the fungus. However, his drawing of a diseased tuber covered with open pustules is very suggestive of powdery scab. No inoculation experiments are reported.

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[1] Accepted for publication May 10, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 294.

Pethybridge (*8*) observed the disease in Ireland and gave it the name of skinspot. Exposed cut surfaces of the pustules, when subjected to a moist atmosphere, produced a strong growth of a fungus identified as *Spicaria solani* Hart. He states that—

> no cultures or infection experiments have been made in connection with this "skin-spot" and, therefore, it can only provisionally be regarded as caused by Spicaria.

Milburn and Bessey (*5*, p. 90–91) attributed an apparently identical disease which they called "blotches" or "pimples" to an organism referred to by them as *Spicaria nivea*. They obtained this fungus in every culture made by planting the inside of the pimples but failed to reproduce the disease by inoculation of healthy tubers with it.

A few years later Güssow (*2*), who previously worked with Carruthers, discarded the fungus theory of skinspot altogether and attributed it to unfavorable storage conditions which interfere with normal respiration of the resting tubers and result in the production of the symptoms of skinspot. He drew his conclusion solely from the observation that this trouble invariably occurred in badly ventilated storage cellars but reports no experimental work along this line.

About the same time O'Brien (*6*), describing Rhizoctonia injuries to potatoes in Scotland, included in his illustrations a photograph (*6*, Pl. V) which in all its characteristic features may be regarded as representative of skinspot. The author, however, uses it as an illustration of an injury caused by *Rhizoctonia crocorum* (Pers.) DC. and states in the text:

> If such an affected tuber be moistened with water, numerous small blackish areas can generally be seen immediately below the surface which cause the skin of the potato to be elevated at such points. These areas are sclerotia.

Apparently without any connection with the English work on skinspot, Wollenweber (*10*) recently published in Germany a pamphlet on potato scabs in which he describes a disease named by him "pustelfäule" and illustrates it with a photograph of the affected potato tuber which leaves no doubt as to the identity of this trouble with the English skinspot. This "pustelfäule," according to Wollenweber, is caused by *Phoma eupyrena* Sacc. He observed the mycelium of this fungus in the diseased tissues and obtained the organism in the majority of his isolation cultures. Further, upon inoculation of healthy tubers in needle pricks he produced "similar spots." He states very clearly that Spongospora was altogether absent in the diseased tissues.

The most extensive and quite recent study of skinspot was made by Owen (*7*). While using for the isolation work only those spots which had the skin unbroken, the author obtained in every culture a similar fungus which is described and named *Oospora pustulans*. In sections of the spots, the fine mycelium of this fungus, it is said, is plainly seen among the dead cells of the potato tissue. The hyphae are very numerous and for the most part hyaline early in April, when the fungus appears to be most vigorous, but later in the season they are very few and mostly brown. Skinspot, according to Owen, is a disease which develops in storage and is not usually visible until the early spring.

In concluding this bibliographical review it may be of interest to mention that the same disease has been locally known in British Columbia as pit Fusarium. The name itself explains its raison d'être—the disease is commonly found in pits and is believed by some to be caused by a species of Fusarium, presumably *Fusarium radicicola*. This erroneous opinion was based on a certain external similarity of skinspot pustules with some initial stages of the lenticel infection with the blackrot fungus as figured

by Pratt (*9*, Pl. 34, fig. 2–3), and also the occasional presence of *Fusarium* spp. in the pustules.

None of the workers mentioned in the preceding paragraphs gave conclusive proof for the support of their respective opinions as to the cause of skinspot. Their conclusions were based mainly on the presence of fungus hyphae within the dead cells and on subsequent cultures of the diseased tissues. The predominant organism in these cultures was assumed to be the cause of the trouble. According to the work of Owen (*7*) it was apparently *Oospora pustulans* in England, while according to the account of Wollenweber (*10*), it was evidently *Phoma eupyrena* in Germany. Güssow (*2*) and O'Brien (*6*) report no experimental work whatever. Carruthers (*1*) and Pethybridge (*8*) confined themselves to microscopic observations only. Milburn and Bessey (*5*) made inoculations of healthy tubers with *Spicaria nivea* but failed to reproduce the disease. Wollenweber (*10*) is content that he produced "similar spots" with *P. eupyrena*, but we know that an insertion of bits of mycelium of almost any facultative parasite in the needle pricks of tubers may result in the formation of brown pimples, externally resembling many a similar pathological condition. He gives no photographs and no description of the artificially produced Phoma spots by which others could judge as to the extent of their similarity with the skinspot pustules.

Owen (*7*) conducted a series of experiments both in the laboratory and in the field, which are described in sufficient detail. The results, however, were either entirely negative or not decisive. Only in one experiment a "small number" of tubers inoculated in the field developed the typical skinspot after they were stored in a cellar from lifting time until April. It should be noted that this experiment was carried on in unsterilized soil in the field, and that—

> much blight was present and a large number of the tubers had to be discarded.

It is not at all impossible that the latter fact may account for the absence of spots in one of the inoculated varieties and, perhaps, in the controls as well. On the other hand, the presence of "a few positive results" which became evident only in April after several months of storage in a cellar can hardly be regarded as proof that this small subsequent development was actually due to the artificial inoculation in July of the preceding year. The possibility of contamination either in the field during the growing period or later in storage is not at all excluded. It is not surprising, therefore, that the author arrives at the conslusion that—

> it will be necessary to wait another season before the results can be confirmed or amplified.

As yet, no further reports have been issued and the work, apparently, has been discontinued, at least temporarily.

TRUE NATURE OF SKINSPOT

While all the alleged causes of the skinspot disease lack convincing proof, a comparative examination of a large number of the affected tubers cannot fail to disclose a great similarity of these spots to the closed-pustule stage of powdery scab. The filamentous fungi invading the tissues of the pustules unquestionably complicate the conditions. Yet, whatever is the effect of this subsequent invasion, there is sufficient evidence that the original cause of this injury is the same as in powdery scab, ascribed to *Spongospora subterranea* (Wallr.) Johns. This evidence is presented hereunder.

MICROSCOPIC CONDITIONS

Kunkel (*3*) has observed the presence of the Spongospora plasmodia in the shrunken areas around the old sori. These the writer has not been able to detect in his sections of the skinspot pustules. The mature spore balls, naturally, are not to be found in the immature sori. Wollenweber (*10*) states that in his material "Spongospora was absent altogether." He does not explain what particular stage of Spongospora was sought in the "pustelfäule" spots or by what methods its absence was ascertained. The difficulty of demonstrating the plasmodium in the pustules of skinspot may find its explanation in the fact that at certain stages of its development it may, apparently, disappear from the spaces it has formerly occupied. This phenomenon has been noted by Kunkel (*3*) even in very young sori. He states:

> Just beneath the epidermis can be seen the dark intercellular spaces which were previously occupied by the infecting plasmodium.

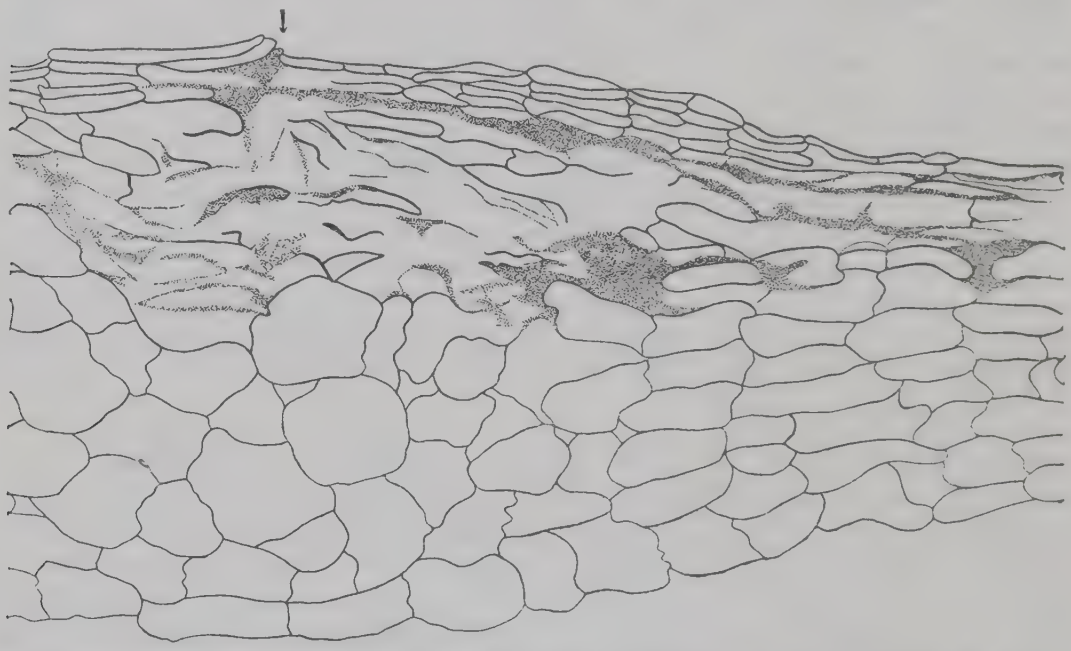

FIG. 1.—Semidiagrammatic drawing of a section through a typical skinspot pustule, showing the tapering spread of the infection between the cells. In several cases the infection has penetrated into the cells. A mass of broken-down cell walls is shown in the center of the spot. Some cells are slightly enlarged. The point marked by the arrow is probably the place of origin of infection. No hyphae have been observed in this spot.

(See also his plates XXXIX, XLI, and XLII.) Aside from this the general appearance of the vertical sections through the raised pimples of skinspot is very characteristic of the condition within the closed sorus of powdery scab (fig. 1). The center is usually filled with a disorganized mass of broken-down cell walls. This débris is surrounded on all sides by darkened intercellular spaces, indicating the path of infection. The infection spreads between the cells in the form of characteristic tapering projections. Occasionally a portion of the cell wall is destroyed and the infection extends into the cell. In some sections hypertrophied cells may also be observed. Fungus hyphae are entirely absent in a certain number of the pustules, which is fully in accord with the results of isolations (Table 1). Such spots may occasionally yield bacteria, but often when transferred to plates they remain sterile. In certain other spots the mycelium, although present, may be found only in very small quantity, dissociated from the apparent course of infection and obviously out of proportion to the area presumably killed by this mycelium.

The formation of new cork in the skinspot pustules on the border line of the diseased and the healthy tissues is also very significant and contradictory to the supposed progress of the disease. This process of cork formation is not invariably associated with the spots, though it may be seen in a considerable number of the specimens. Owen (7) gives a drawing of a section through a pustule of skinspot which shows distinct layers of new cork below the affected areas. Doubtless this phenomenon signifies that the parasite, after killing several layers of cells unhindered in its attack, finally ceased its activities, after which the host tissues erected a barrier between the dead and healthy regions. It is the course diametrically opposite to that which we would expect in *Oospora pustulans*, which is said to be invisible at lifting time but develops a considerable injury toward spring, or about the month of April. It should, therefore, at this time of the year be in its most active stage and not in a dormant state. On the other hand, the phenomenon of cork formation is quite in harmony with the progress of powdery scab. The active parasitic stage of the latter develops in the field during the growing period of the tuber. As observed by Kunkel (3), a certain degree of saprophytic activity of the fungus plasmodium develops in storage. This secondary infection results in dry-rot areas around the sori. However, it is, apparently, very slight with some spots and may be entirely checked with others. The cicatrisation in such cases is obvious.

ABSENCE OF THE SKINSPOT FUNGUS

During the years of 1920 and 1921 the writer had an opportunity to examine and study an abundance of material showing skinspot infection. This material was secured not only from England but also from other countries. It is believed, therefore, that a comparison of cultures isolated from this material will be of special value and interest. The various samples from which isolations were made are as follows:

1. Several tubers from British Columbia, crop of 1919, received in spring of 1920. Pustules only slightly raised above the surface of the tuber.

2. Five tubers from Dr. A. D. Cotton, England, crop of 1919, received in spring of 1920; sent upon request as representative samples of the skinspot disease.

3. Several lots of wart-resistant varieties shipped from England for work in Pennsylvania, crop of 1919, received in the winter of 1919–20. (Pl. 1, A to H, and Pl. 2, C and D, show some of these tubers.)

4. Tubers from various consignments of Danish potatoes received at the port of New York, crop of 1919, collected in early spring of 1920, (Pl. 2, B, shows one of these tubers.)

5. A sample of potatoes from New Castle, England, secured by Federal Horticultural Board inspectors in the spring of 1920.

6. A sample of potatoes from Edinburgh, Scotland, secured by Federal Horticultural Board inspectors in the spring of 1920.

7. A sample of potatoes from Ireland, secured by Federal Horticultural Board inspectors in the spring of 1920.

8. Two samples of the diseased tubers sent by Prof. J. W. Eastham, British Columbia, crop of 1920, received in the winter of 1920–21.

9. A number of tubers from the progeny of the infected English varieties (sample No. 3) planted in Pennsylvania in 1920, isolations made in the spring of 1921.

10. Tubers showing closed-pustule stage of powdery scab collected by the writer at Vancouver, B. C., in the fall of 1920. They were thoroughly washed after digging and stored for winter in a laboratory refrigerator. Isolations were made in March, 1921. (One of these tubers is shown in Pl. 3, A.)

11. Irish Cobbler tubers from Maine, crop of 1920, showing immature sori of powdery scab. Isolations were made in the winter of 1920–21. (One of these tubers is shown in Pl. 3, B.)

12. The same material as in the preceding sample, but isolations were made from mature sori.

Isolations were made from each of these samples and the results are presented in Table I.

TABLE I.—*Results of isolations from the skinspot pustules and the sori of powdery scab*

Number of the sample.	Number of spots from which isolations were made.	Number of spots which yielded no growth.	Number of spots which yielded *Oospora pustulans*.	Number of spots which yielded miscellaneous fungi and bacteria.	Remarks.
1	27	3	1	23	6 spots yielded *Fusarium* spp.
2	60	3	49	8	3 out of 5 tubers (42 spots) yielded exclusively *Oospora pustulans*.
3	15	5	6	4	No isolations were made from the open pustules shown on the photograph.
4	24	2	10	12	Samples showed no mature sori of powdery scab.
5	6	0	5	1	Sample similar to the preceding one in appearance.
6	6	0	3	3	Do.
7	6	0	6	0	Do.
8	34	2	6	26	1 sample (12 spots) yielded no culture of *Oospora pustulans*.
9	60	0	0	60	The predominant culture in this case was *Colletotrichum atramentarium* (Berk. and Br.) Taub.
10	12	10	0	2	1 *Phoma* sp. and 1 *Verticillium* sp.
11	12	7	0	5	Only 1 sorus yielded *Phoma* sp.
12	31	3	0	28	16 sori yielded *Phoma* sp. and the remainder miscellaneous fungi.
Total of 1 to 9 (undoubted skinspot).	238	15	86	137	*Oospora pustulans* 36.1 per cent.
Total of 2, 3, 5, 6, and 7 (English material).	93	8	69	16	*Oospora pustulans* 74.2 per cent.
Total of 1, 4, 8, and 9 (American and Danish material).	145	7	17	121	*Oospora pustulans* 11.72 per cent.

These results of isolations made from various lots of material in 1920 and 1921 show that *Oospora pustulans* is by no means the only invader of the skinspot pustules, since it comprised 36.1 per cent, or slightly above one-third, of the total isolations from the undoubted skinspot material.

Nor is *Phoma* sp. alone in attacking the *Spongospora* sori in Maine. In addition to a certain proportion of sterile plantings, that is, those which yielded neither fungi nor bacteria, a considerable percentage of organisms other than *O. pustulans* was obtained from authentic skinspot material. Especially striking is the complete absence of the supposed skinspot fungus from the typical pustules of the disease developed on potatoes grown in Pennsylvania. The largest number of *O. pustulans* cultures was isolated from the tubers grown in the British Isles (69 out of the total number of 86 *O. pustulans* cultures, or 74.2 per cent, of all the plantings made from English material). A somewhat smaller proportion was obtained from Danish specimens and a still smaller one from the samples received from British Columbia. None was isolated from the skinspot pustules which developed on potatoes grown in Pennsylvania.

It is not surprising that plantings of the diseased tissues did not yield *Spongospora subterranea*. So far only one worker (*3*) has reported success in culturing this organism; and, furthermore, these cultures were obtained by germinating the mature spore balls which are not yet present in the closed sori.

That a considerable number of the true powdery scab sori are likely to become invaded by some secondary parasite is not a new discovery. Previous studies of this disease in Maine revealed the fact that even in good storage conditions 30 to 75 per cent of the tubers infected with powdery scab suffer from a secondary rot due to *Phoma tuberosa*. The colored plate accompanying the paper cited here (*4*) shows two tubers with a number of closed sori. The latter have a depressed border and a raised center corresponding to the characteristics noted in connection with skinspot in England; but the organism responsible, at least in part, for this condition in the latter country is apparently *Oospora pustulans*. In Germany, Wollenweber's (*10*) work indicates, that a similar effect is produced by a species of Phoma different from that occurring in Maine. The principal invader of the skinspot pustules in Pennsylvania appears to be *Colletotrichum atramentarium*. This predominance in this connection of a certain species of fungus in a given country is an interesting phenomenon worthy of further study. It raises a question as to the rôle of climatic conditions in developing an intricate relationship within the fungus world, and in this case relationship between the powdery scab parasite and the secondary invader. It does not, however, solve the problem of the original cause of skinspot. It has long since become a commonplace in the science of plant pathology that the association of a certain fungus with the dead host tissue does not necessarily signify that it is the primary cause of death. It may as well denote any one of three other possibilities, namely, a secondary invasion of a parasitic nature, a saprophytic existence on the dead stratum, or a parasitic attack on the primary parasite. *Fusarium* spp. isolated by the writer from the skinspot pustules are, undoubtedly, secondary parasites, while such miscellaneous fungi as *Penicillium* sp. or *Actinomyces* sp. probably live there saprophytically. The part which is actually played in the potato tissues by *O. pustulans* as well as that by *Phoma eupyrena* or *C. atramentarium* remains as yet to be shown. The writer's laboratory inoculations of healthy potato tubers with *O. pustulans*, like those reported by Owen (*7*), gave entirely negative results.

It is not difficult to culture *Oospora pustulans* whenever the spots are actually invaded by this fungus. Therefore, the disagreement of the various investigators as to the cause of skinspot does not by any means

signify their inability to secure this organism in culture, were it always present in the diseased tissues. It is merely a reflection of the actual state of things. Their conclusions were determined mainly by the prevalence of one or the other semisaprophytic fungus in their cultures or by the absence of any apparent growth, as the case might have been. So far as the primary cause of the trouble is concerned, they were dealing with an organism which is far from being readily culturable by our usual laboratory methods.

VARIOUS STAGES ON THE SAME TUBER

While it is possible to single out many tubers which are covered only with the closed pimples to which the name skinspot is applied, yet in the examination of large quantities of infected material one is likely to find a certain number of specimens, varying perhaps with the seasonal conditions, which show all gradations in the development of the sori. It is rather difficult and sometimes impossible to determine whether the closed pustules in such cases should be classed as skinspot or powdery scab (Pl. 1, E to H). Like difficulty may be encountered in an examination of some unquestioned powdery scab material, grown, for instance, in Maine or Canada (Pl. 3, A, B, C).

An interesting question arises as to why in certain years there is an abundance of immature sori of the Spongospora scab. Theoretically speaking, this condition may be due either to an early check in the development of the disease or to a late infection. Both may be brought about by drouth and aided by the varietal response of the tubers. It is probable that late varieties with a later formation of tubers are more likely to show undeveloped powdery scab pustules, especially under adverse conditions.

During the latter part of September, 1920, the writer visited Sea Island and Lulu Island in British Columbia. A number of fields were examined on which it was said skinspot infested crops were produced in the preceding years, but no signs of the disease were noticed. Neither was the well-developed stage of powdery scab common. The local workers explained that the last two seasons had been very dry and in consequence powdery scab was practically eliminated. On the other hand, 1919 was the year when skinspot made its pronounced appearance and was noticed in abundance by United States inspectors and pathologists.

An early stage of Spongospora infection is usually not conspicuous at harvesting. The infected spots are not dark in color until they are exposed to the drying which takes place after lifting. During the long period of storage the tubers gradually lose moisture and shrink. The hard, dry, dark pustules then become elevated, especially when they become invaded by saprophytic or semisaprophytic organisms.

DISCOLORATION AND ARRANGEMENT OF THE SKINSPOT PUSTULES

Upon lifting the epidermis the majority of the spots show a peculiar chocolate-brown or olive-brown discoloration of the affected areas. This discoloration has frequently been described in the literature as very characteristic of powdery scab. It is not known to be so constantly associated with any other disease of the potato tuber. The arrangement of the pustules over the skin is likewise very suggestive of the Spongospora scab. Although often scattered without any definite order (Pl. 2, D), as is also the case with indisputable specimens of powdery scab

(Pl. 4, A), they are frequently arranged in a certain peculiarly regular manner, as for instance, in the form of bands or stripes (Pl. 1, A; Pl. 2, B, C), rings (Pl. 1, C, D) and aggregations, particularly at the stem end (Pl. 2, A; Pl. 3, A). All this is very typical of powdery scab (Pl. 4, B, C, D).

GEOGRAPHICAL DISTRIBUTION OF SKINSPOT

It is a striking "coincidence" that this disease occurs only in those countries, or in those sections of the countries, where climatic conditions favor the development of powdery scab. It has not yet been reported from those parts of the world where the latter is unknown. Thus, it is prevalent in the British Isles, exists in Denmark and Germany, and is established in parts of Canada. Occasional specimens showing closed pustules were observed in Maine, and they were always identified as the immature stage of powdery scab. It has developed recently to a slight degree in the elevated regions of Pennsylvania. No other similar trouble has been discovered in any other section of the States in spite of a continuous influx of skinspot infected potatoes from Europe. This is exactly the behavior of powdery scab. The writer planted a large quantity of *Oospora pustulans* material at Arlington Farm, Va., in 1920, but no spots were observed on new tubers either at harvest time or in the spring of 1921. The same negative results were obtained with tubers planted in the greenhouse at Washington, D. C., and grown in various kinds of soil and under various conditions of moisture. Although the powdery scab sori may develop in Washington greenhouses, this is known to take place only when tubers with the mature spore balls are planted. It has not yet been ascertained whether the organism in the closed pustule stage can readily, if at all, become a source of infection of the new crop.

SUMMARY AND CONCLUSIONS

Evidence is submitted in the preceding paragraphs which show that:

(1) Neither *Oospora pustulans* nor any other filamentous fungus or environmental conditions mentioned by previous authors in connection with skinspot are proved to be the primary cause of the disease.

(2) *Oospora pustulans* is prevalent in England, and probably in some geographically proximal countries, but is either rare or entirely absent in the skinspot material grown in America.

(3) The various peculiar characteristics of the skinspot disease, as well as its geographical distribution are fully in accord with our present knowledge of powdery scab.

(4) Certain of these peculiar features can not be satisfactorily explained apart from the identity of the two diseases.

(5) The closed or immature sori of powdery scab present no apparent difference from the pustules of skinspot.

(6) The fungi associated with the spots in question are mainly secondary invaders developing during the storage period.

(7) These secondary invaders do not belong to one species, nor even to one genus, but vary with the country, their prevalence being determined perhaps by climatic conditions and the flora of the soil.

(8) Their presence in the sori of powdery scab is altogether unnecessary to give them the appearance of skinspot.

This evidence leads us to the conclusion that the skinspot pustules are essentially and primarily the closed or immature sori of powdery scab.

They are, however, frequently invaded during the period of storage by various filamentous fungi, in part saprophytes. In the light of this conception of skinspot we can understand the failure of this disease to spread in the United States in spite of numerous and continuous importations of infested material. We may further rest assured, so far as our knowledge of powdery scab goes, that the skinspot stage of the latter can never become a serious menace to the potato industry in this country. The term skinspot has no right to existence, except perhaps for practical convenience as a designation of a certain stage of powdery scab.

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PLATE 1

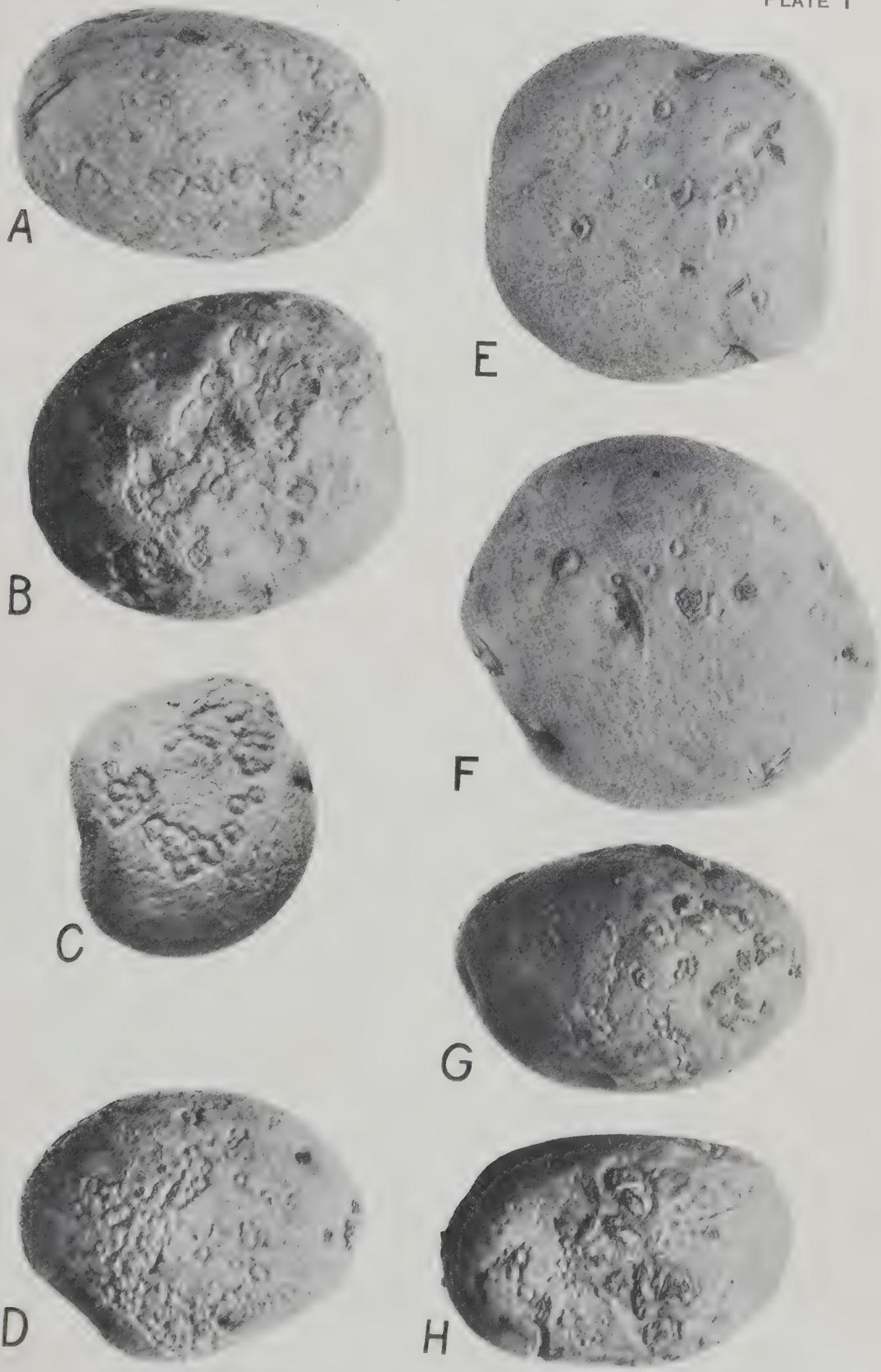
Various stages and arrangements of the typical skinspot pustules. Some tubers show mature sori of powdery scab also. All eight specimens were grown in England.

A.—Stripe arrangement of the pustules.

B.—Perfectly flat spots.

C, D.—Circles of the sori.

E to H.—Mixed stages of maturity of sori.


A
B
C
D
E
F
G
H

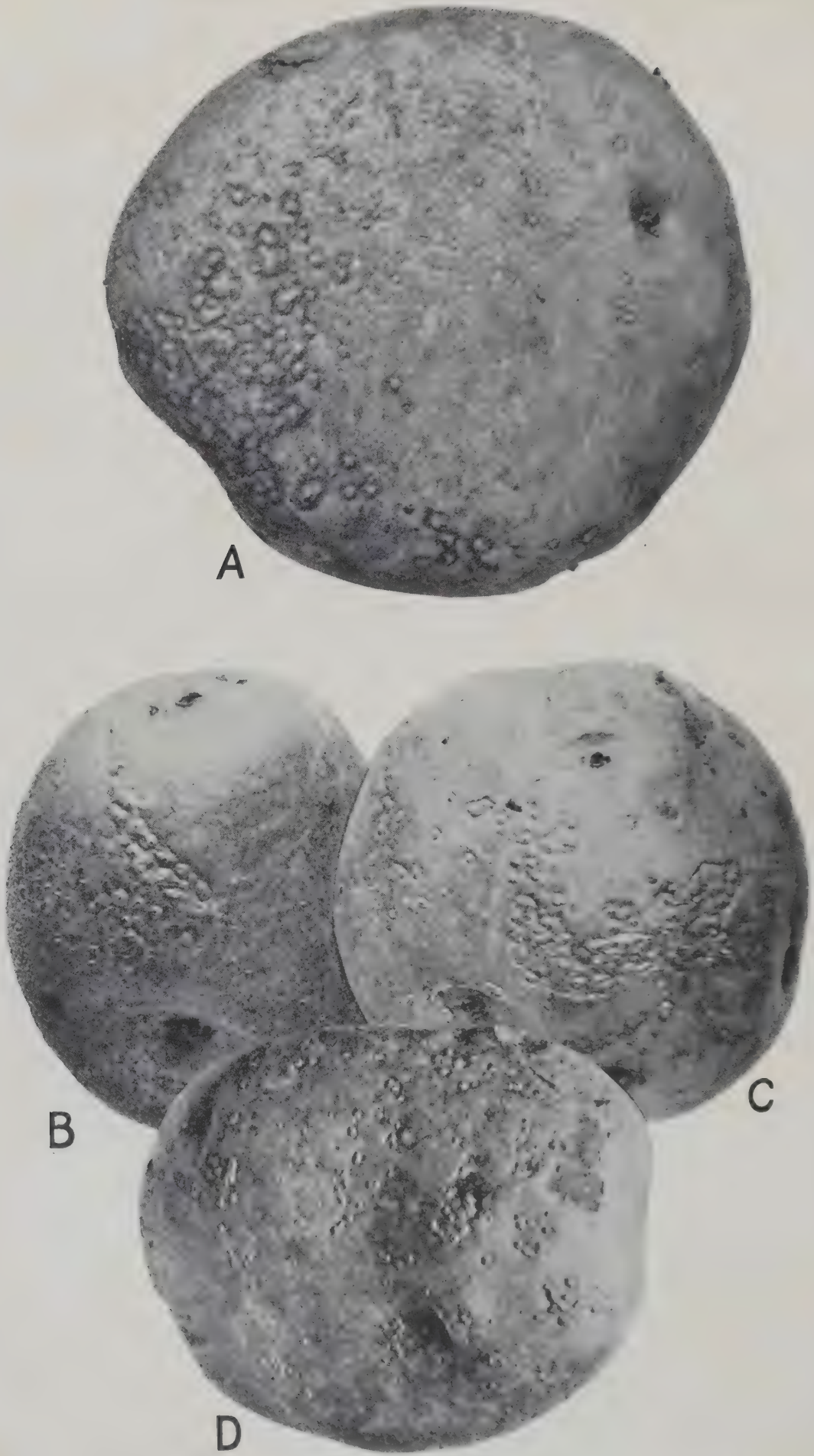

A
B
C
D

PLATE 2

Closed and half-closed sori.

A.—Aggregation of sori at the stem end of a tuber from Scotland.
B.—Stripe arrangement on tuber taken from a consignment of Danish potatoes.
C.—Semicircle of pustules.
D.—Promiscuous distribution of pustules over the surface of the tuber.
C and D were received from England.

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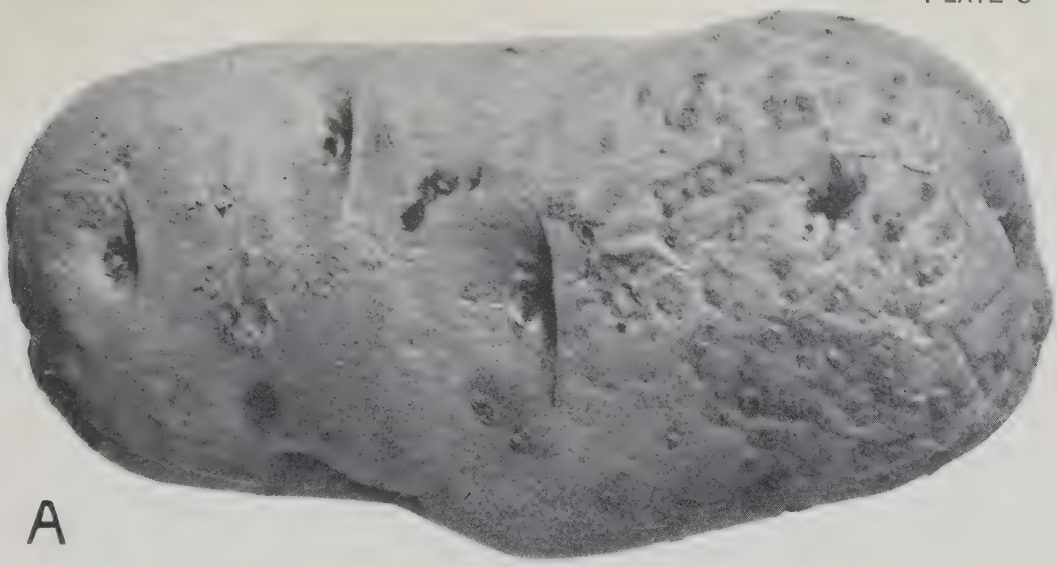
PLATE 3

Tubers showing immature closed and half-closed sori of powdery scab, most closely approaching the perfect skinspot stage: A, from British Columbia; B and C, from Maine.



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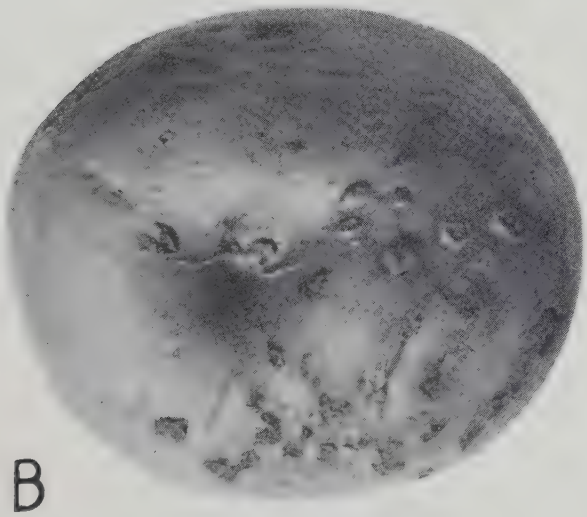

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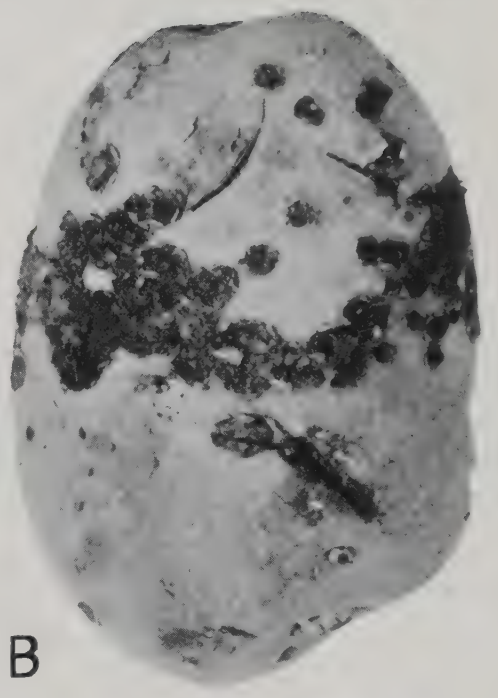
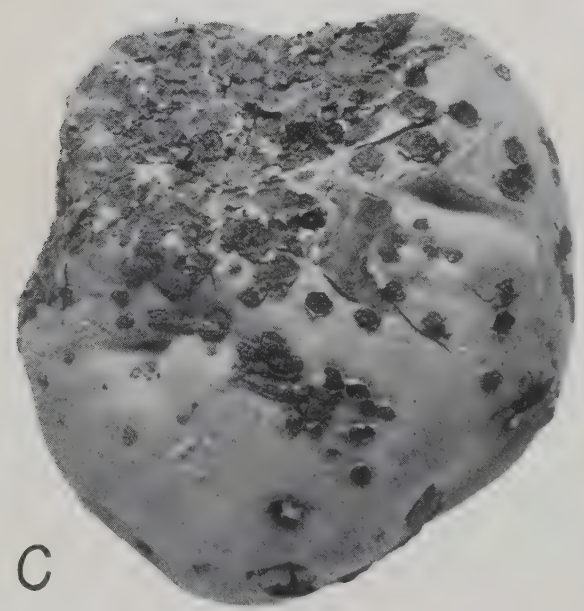
C.

A


B


C

D

PLATE 4

Typical open sori of powdery scab, illustrating the distribution of the pustules: A, promiscuously scattered over the surface of the tuber; B, in a semicircle; C, in an aggregate at the stem end; D, as a band encircling the tuber.

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USE OF ALTERNATING TEMPERATURES IN THE GERMINATION OF SEEDS[1]

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INTRODUCTION

The helpful use of daily alternations of temperatures in the germination of certain seeds grew out of observed differences in germination in light as compared with that in darkness, and the resulting controversy as to whether the favoring effect of light was due to the heat rays or was photochemical in nature.

Cieslar (*3*)[2] in 1883 secured better germination of *Poa nemoralis* in yellow (therefore warm) light than in white light, although violet (therefore cold) light gave no better germination than was secured in darkness. Pickholz (*19*), working with *Poa pratenis*, later reported similar results which indicated that the heat rays were more effective in germination than the light rays. While this conclusion has not proved to be true with all light-sensitive seeds, it has with some kinds; and the investigations which followed Cieslar's work led to the definite policy of germinating many kinds of seeds with a regular daily alternation of temperature. Usually about 20° C. has been used as the lower temperature and about 30° as the higher temperature, the higher temperature being maintained for from four to eight hours each day. Many other alternations have been used experimentally with good results.

The year following Cieslar's publication Von Liebenberg (*16*) showed that *Poa pratensis* germinated better when kept at 20° C. for 19 hours and at 28° for five hours of each day than at either 20° or 28°, constantly maintained, and that this alternation of temperatures took the place of exposure of the seed to light. Since then others (*1*, *2*, *6*, *7*, *8*, *9*, *11*, *15*, *17*, *18*, *19*, *20*, *21*) have shown a favorable effect of temperature alternations upon the germination of many kinds of seeds. Usually a daily alternation has been used. Heinrich (*13*), however, showed that a very beneficial effect was produced in some cases by a definite temperature change once in five days. Lehman (*15*) argued that a similar favorable effect was produced by temperature changes without definite or daily alternations, and Gassner (*6*) showed that only a few hours at a low temperature followed by a single change to a higher temperature induced maximal germination of seeds of *Paspalum dilitatum*, which would not germinate well without previous exposure to the low temperature.

[1] Accepted for publication May 10, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 331-332.

Haberlandt (*10*) and Eidam (*5*) have suggested temperature effects upon the water intake of seeds as a possible cause of the favorable effect of temperature alternations upon germination. This hypothesis seems hardly tenable, since certainly some and probably most seeds whose germination is favored by temperature alternations take up at any temperature more water than the minimum required for germination. Von Liebenberg (*16*) suggested that the reserve materials made available at any given temperature were wholly or largely used up in respiration, but that a slight surplus, becoming soluble at higher temperatures, is available for growth when the temperature is lowered with consequent reduction in the intensity of respiration. Gassner (*7*) considered the effect of temperature alternations upon the germination of *Chloris ciliata* to be entirely a matter of oxygen relations. In line with Gassner's conclusion Vanha (*21*) called attention to the fact that differences in temperature between the different parts of the seed, the germinating bed, and the outer air following a sudden temperature change cause different gas densities which may set up lively gas movements leading to removal of CO_2 and renewal of oxygen—conditions favorable to increased respiration and probably to germination.

Probably none of these hypotheses can furnish a complete explanation of the effect of temperature alternations, but they point the way for an attack upon the problem. A detailed study of the respiration and internal changes of the seeds at different temperatures would probably help in its solution. The respiratory quotient of dormant apple seeds decreases with decreasing temperature, and vice versa. If this is true of dormant embryos generally, there may be at low temperatures an accumulation and actual metabolism of oxygen in a form which becomes immediately available for the inception of growth processes when the seeds are placed at the higher temperature. This hypothesis is in some respects the converse of that of Von Liebenberg (*16*), but it does not stand in opposition to that of Vanha (*21*) or to that of Gassner (*7*).

The purpose of the present paper is not to discuss the possible explanation of the effects of temperature alternations upon germination but merely to show somewhat more in detail the nature and extent of such effects.

EXPERIMENTAL WORK

Several years ago seeds of carrot (*Daucus carota* L.), celery (*Celeri graveolens* (L.) Britton), parsley (*Petroselinum hortense* Hoffm.), and parsnip (*Pastinaca sativa* L.) were tested for germination at 15°, 20°, 25°, 30°, and 35° C., and with every possible alteration between these temperatures in which the higher temperature was maintained 7½ hours and the lower temperature 16½ hours of each day—a total of 5 constant temperatures and 10 temperature alternations. The temperature alternations were accomplished by transferring the trays with their load of seeds from one germination chamber to another and back again each day.

Under nearly all of these 15 temperature conditions, germination tests of seeds of the following grasses were made: Timothy (*Phleum pratense* L.), awnless brome grass (*Bromus inermis* Leyss.), perennial rye grass (*Lolium perenne* L.), Italian rye grass (*Lolium multiflorum* Lam.), meadow-fescue (*Festuca elatior* L.), orchard grass (*Dactylis glomerata* L.), redtop, (*Agrostis polustris* Huds.), Kentucky bluegrass (*Poa pratensis* L.), and Bermuda grass (*Capriola dactylon* (L.) Kuntze).

In these germination tests it was not possible with the equipment available to control the temperatures absolutely, but except for 15° and 20° C. the fluctuations were very slight. Even with these lower temperatures the control was sufficiently accurate to give reasonably reliable results, as will appear later.

Seeds of celery and redtop were tested on top of four thicknesses of moistened blotting paper. Kentucky bluegrass and Bermuda grass seed were tested with the Jacobson apparatus (*4, p. 33*). The other kinds were tested between moistened blotting papers, two thicknesses below and two above.

The following year a study was made of the actual progress of temperature changes in the interior of the germinating chambers in a variety of temperature alternations, and these temperature changes, with the resulting effects upon the germination of a number of kinds of seeds, were compared with data obtained from field experiments.

About two years later a number of kinds of flower seeds were tested at very accurately maintained constant temperature from 15° to 30° C. with a large number of temperature alternations. Some kinds were tested also in an ice box. The kinds studied were balsam (*Impatiens balsamina* L.), California poppy (*Eschscholtzia californica* Cham.), candytuft (*Iberis amara* L.), cosmos (*Cosmos bipinnatus* Cav.), belvedere (*Kochia scoparia* (L.) Schrad.), larkspur (*Delphinium ajacis* L.), marigold (*Calendula officinalis* L.), mignonette (*Reseda odorata* L.), nasturtium (*Tropaeolum majus* L. and *T. minus* L.), pansy (*Viola tricolor* L.), petunia (*Petunia hybrida* Hort.), Chinese pink (*Dianthus chinensis* L.), poppy (*Papaver* spp.), portulaca (*Portulaca grandiflora spelndens* Hort.), snapdragon (*Antirrhinum majus* L.), sweet pea (*Lathyrus odoratus* L.), and zinnia (*Zinnia elegans* Jacq.). The larger seeds were tested between wet blotters or wet canton flannel and the smaller ones on top of wet blotters.

More recently a careful study was made of the germination of Johnson grass seed (*Holcus halepensis* L.) under accurately controlled temperature conditions, moistened blotting paper disks in 100-mm. Petri dishes being used as a germinating bed.

In the germination tests of each series duplicates of 100 or 200 seeds each were used. The seeds used were fully after-ripened except as indicated later in certain tests of Johnson grass seed. The tests whose results are presented in this paper are typical examples of a large number of tests of the same kinds of seed.

The results of the first and third series of tests will be presented briefly and then the results of the tests of Johnson grass seed will be given more in detail, reserving the study of the alternations and comparison with field data until last. On account of the nature and complexity of the data dealt with, the results can in general be presented to best advantage in graphic rather than in tabular form.

RESULTS OF THE EXPERIMENTS

I. SEEDS GERMINATING AT CONSTANT TEMPERATURES

Seeds of timothy, awnless brome grass, both species of rye grass, meadow fescue, carrot, and parsley and all of the flower seeds germinate nearly or quite as rapidly and completely at favorable constant temperatures as with any alternation of temperatures. The most

favorable constant temperature for the different species varied from 15° C. for larkspur and poppy seeds to 22.5° or 25° for petunia seed. Some kinds of seed germinated equally well with a wide range of temperatures, while for others there was a very definite temperature optimum. For instance, larkspur seed germinated much better at 15°, which represents its optimum, than at 17.5°, while belvedere seed germinated with

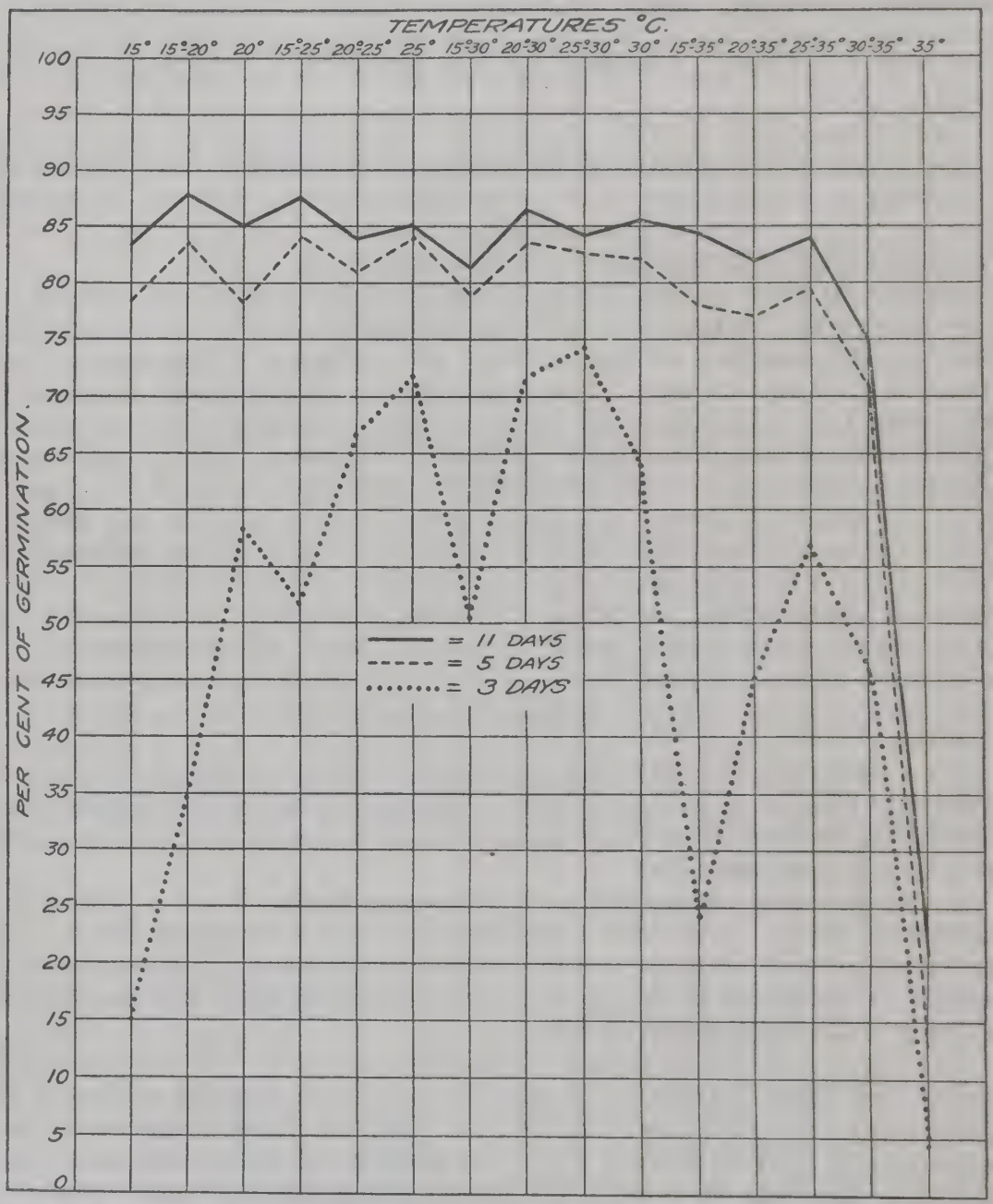

FIG. 1.—Average rates and percentages of germination of two lots of carrot seed under 15 temperature conditions.

equal completeness and with very little difference in rapidity at any temperature from 15° to 30°.

Figure 1, representing the average rates and percentages of germination of two lots of carrot seeds under 15 temperature conditions, illustrates the behavior of those seeds which germinated well at constant temperature and were tolerant of a wide range of temperatures. The figure shows a retarding effect of very cool or very warm temperatures and of alternations of temperature between extremes which are widely

separated from each other. But the differences observed in the early days of the test were evened up toward the end, except with the warm temperature alternation 30° to 35° C. and at 35° constant.

2. SEEDS REQUIRING TEMPERATURE ALTERNATIONS

Seeds of redtop, orchard grass, Kentucky bluegrass, Bermuda grass, parsnip, celery, and Johnson grass required an alternation of temperatures for best germination. Of these, redtop comes nearest to being a constant-temperature germinator and Bermuda grass stands at the other end of the series.

REDTOP

Figure 2 shows the average percentages and rates of germination of three samples of redtop seed. Alternations between extremes 10° C. apart (20° to 30° and 25° to 35°) gave the highest results, with 5° and 15° alternations only slightly lower but still somewhat ahead of the constant temperatures. As with the constant-temperature germinators, so here in the tests with redtop, constant temperatures below or very much above the optimum and alternations 15° wide (20° to 35°) retarded germination in the early days of the test. At the end of the first three days the influence

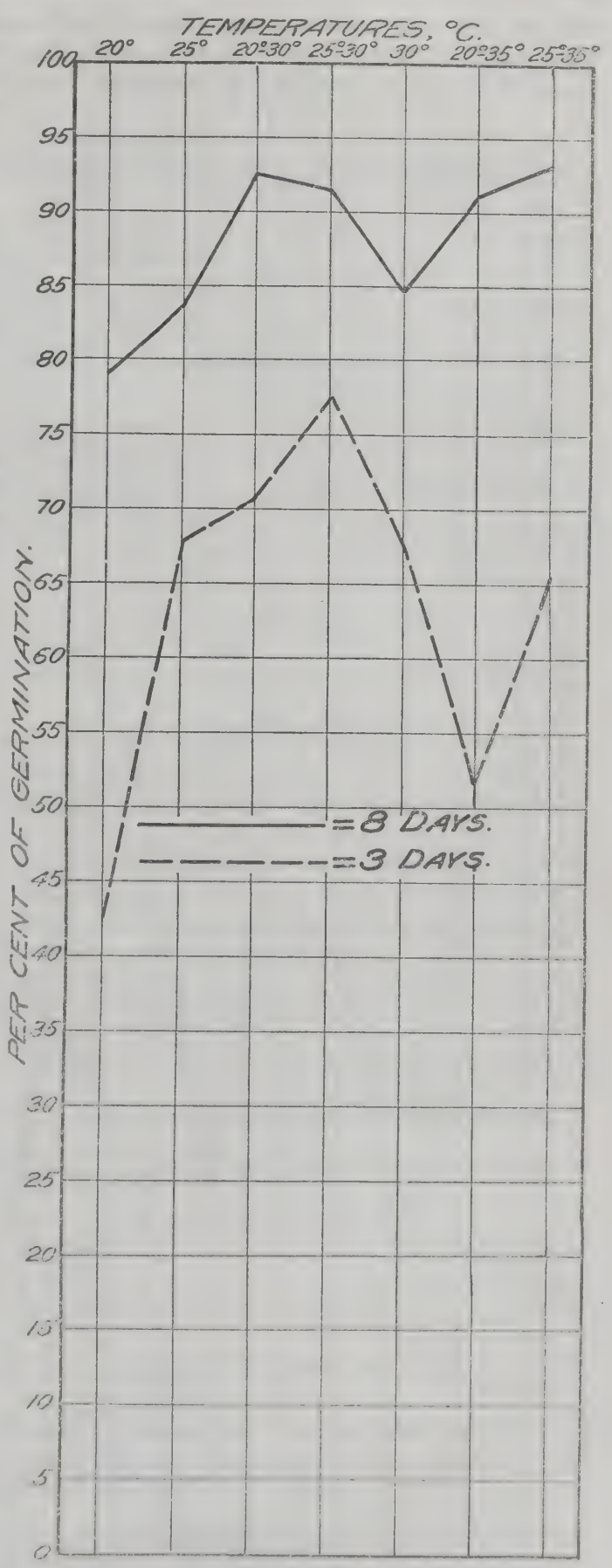

FIG. 2.—Average rates and percentages of germination of three lots of redtop seed under seven temperature conditions.

of the actual mean temperature seems to predominate, except with the wide alternation 20° to 35°, over the influence of alternations as contrasted with constant temperatures; and it is only in the later days

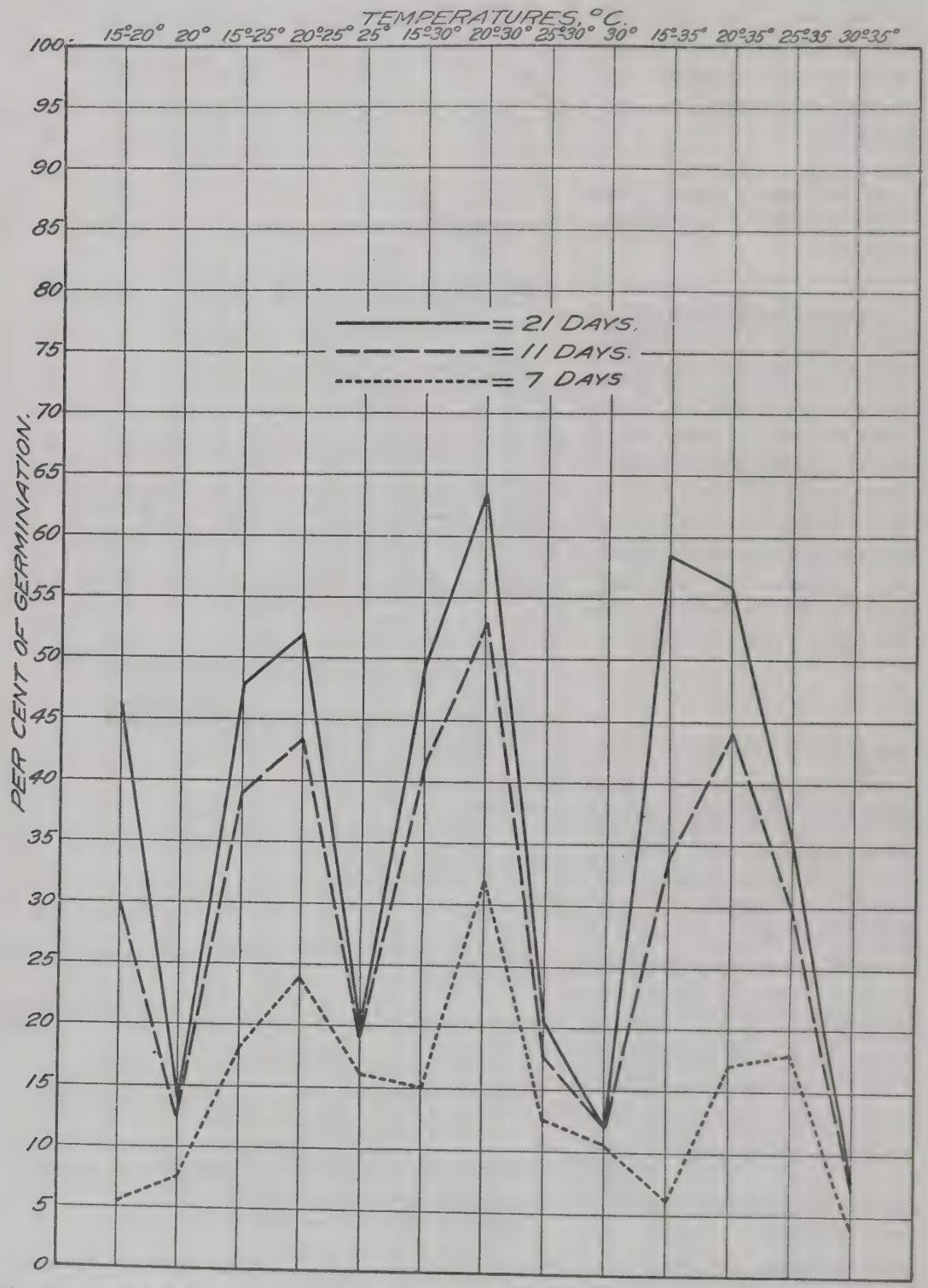

FIG. 3.—Average rates and percentages of germination of three lots of Kentucky blue-grass seed under 13 temperature conditions.

of the tests that some of the alternations prove their superiority. From the standpoint of rapidity of germination, alternations with 30° are preferable to alternations with 35° as the higher temperature.

KENTUCKY BLUEGRASS

Figure 3 shows the average percentages and rates of germination of three lots of Kentucky bluegrass seed. Here the favorable effect of temperature alternations is strikingly evident and increases with the progress of the test. All of the low points in the graphs, except for the earliest count (seven days), represent germination at constant temperatures or with narrow (5° C.) alternations. A narrow alternation was more effective in a low temperature range (15° to 20° and 20° to 25°) than in a higher temperature range (25° to 30°). This has been found to be true also with other seeds requiring temperature alternations for best germination. An alternation of 15° retarded germination in the early days of the test. The alternations 25° to 35° and 30° to 35° were too warm for good results.

It should be emphasized here that the beneficial effect of temperature alternations can not in any way be referred to a constant temperature equivalent to the mean temperature of the alternation or to the extreme temperatures of the alternation. Thus, temperature alternations with 20° C. as either upper or lower extreme gave much better results than 20° constant, and any alternation gave better results than either of the extremes of that alternation constantly maintained. Again, the germination with the alternations 15° to 30° and 20° to 35° was approximately three times as great as at their constant temperature equivalents, 20° and 25°.

At the same time it is possible to see an effect of mean daily temperature supplementing the more important effect of the temperature changes as such. Since the higher temperature in each alternation was maintained for approximately one-third of each day the mean temperature of each alternation can be represented by adding to the lower temperature one-third of the difference between that and the higher temperature. Table I shows the mean temperatures of the alternations which gave fairly good results, together with the average total percentages of germination. The germination increased regularly with increase in the mean temperature of the alternation up to 23° C. and fell rather abruptly after this mean. There was a similar correspondence between mean temperatures and the germination of Johnson grass seed, but not with any of the other seeds which required alternations of temperatures for best germination.

TABLE I.—*Temperature alternations, corresponding mean temperatures, and average percentages of germination produced in three lots of Kentucky bluegrass seed*

Temperature alternation.	Approximate mean temperature.	Average percentage of germination.	Temperature alternation.	Approximate mean temperature.	Average percentage of germination.
°C.	°C.		°C.	°C.	
15 to 20	16	46	15 to 35	22	58
15 to 25	18	48	20 to 30	23	63
15 to 30	20	49	20 to 35	25	56
20 to 25	21	52	25 to 35	28	35

Kentucky bluegrass is one of the kinds of seed with which Hartleb and Stulzer (*12*) secured much better germination at 30° C. than with an alternation between 20° and 30°. The results here reported, as well as the results of several other investigators, show that Hartleb and Stulzer's results were unreliable and their conclusions false.

The temperature alternation giving best results in these tests (20° to 30° C.) is almost identical in mean temperature with that (21° for 8 hours and 28° for 16 hours) which Vanha (*21*) in his careful and thorough investigation found most advantageous for the germination of this species. Brown (*1*) also recommended an alternation between 20° and 30° for the germination of seeds of this species.

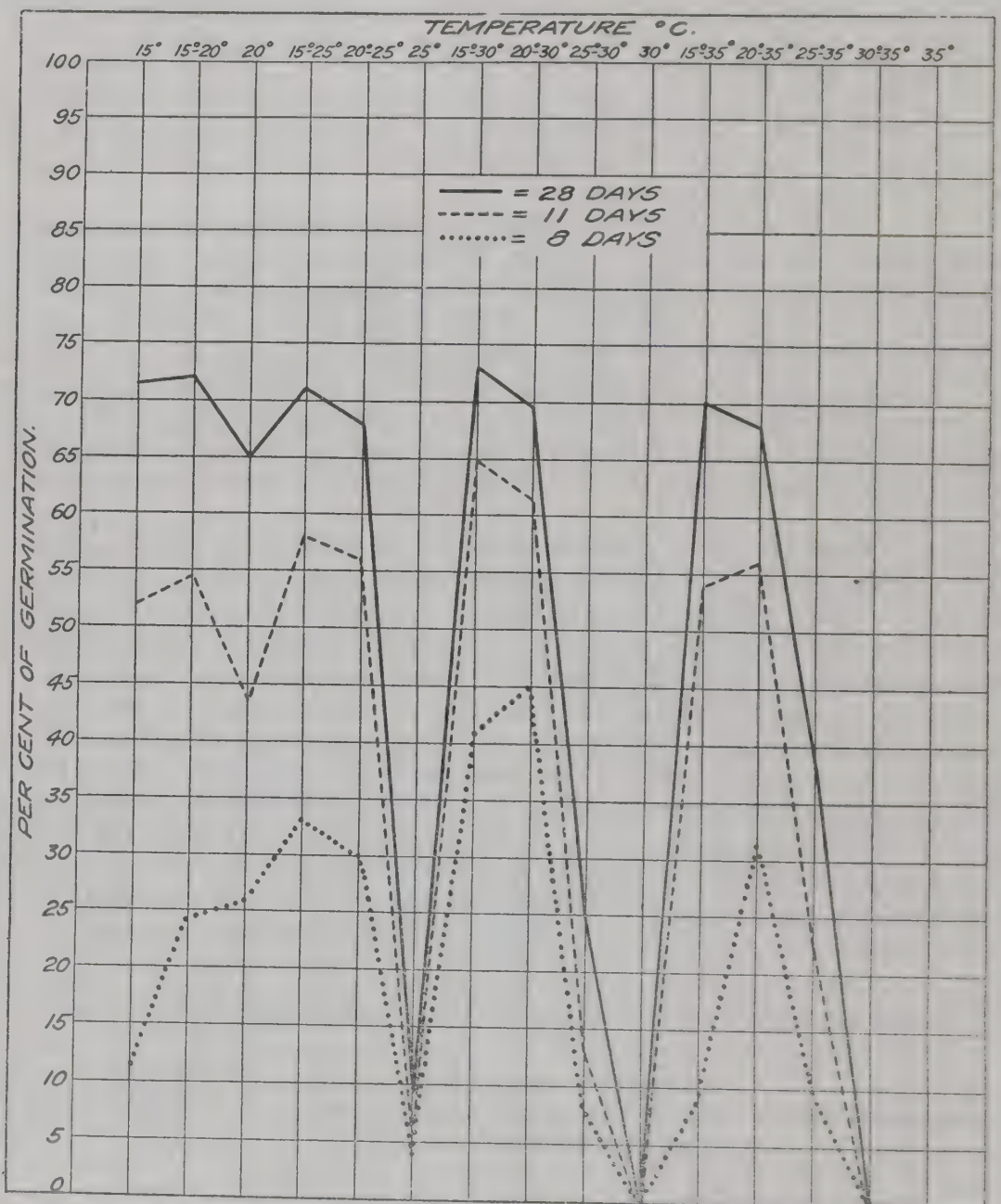

FIG. 4.—Average rates and percentages of germination of three lots of celeryseed under 15 temperature conditions.

CELERY

Figure 4 shows that celery seed germinated almost as completely at nearly constant low temperatures as with temperature alternations, though it germinated more slowly. However, as the germination temperature increased a relatively wide alternation (10° C.) became imperative and still wider alternation (15° or 20°) did no harm. As with Ken-

tucky bluegrass, the favorable effect of the alternations is not referable to the effect of extreme or mean temperatures but is the result of the changes in temperature. In several other tests of celery seed with better control of the lower temperatures, germination was much poorer at 20° and with the alternation 20° to 25°, in comparison with other alternations, than in the tests represented in Figure 4. No other tests were made at 15° or with the alternation 15° to 20°.

The slight fluctuations in the temperature of the cool germinating chambers were no doubt partly responsible for the high percentages of germination at low temperatures; but they certainly were not sufficient to explain the results entirely if the seeds required as wide alternations at low temperatures as at high temperatures and if no other significant factor was involved. Additional tests made at another time indicate, however, that other factors may be involved. Ninety-six simultaneous tests of celery seed from a single unusually sensitive lot were made in different parts of a single chamber, which was heated from below by a gas burner during the several hours of each forenoon and cooled each afternoon by a stream of cold water in the top of the water jacket around the chamber. The upper and lower temperatures were controlled by thermo regulators, and the lower temperature was held for about 15 hours of each day. The seeds in the more rapidly cooled parts of the chamber germinated much more rapidly and completely than those in other parts of the chamber, though the extreme upper and lower temperatures reached were very nearly the same in all parts of the chamber. The actual range in percentage of germination in different parts of the chamber was from 0 to 32 per cent in 7 days, from 0 to 58 per cent in 9 days, from 11 to 81 per cent in 11 days, from 41 to 91 per cent in 14 days, and from 53 to 95 per cent in 21 days. After 21 days the seeds which had occupied the least favorable positions and had germinated most poorly were put in the most favorable positions, with the result that their germination soon increased to equal that of the seeds originally in the more favorable positions.

Similarly, when the celery seed was daily transferred between two chambers constantly maintained at different temperatures and the cool chamber was cooled by a block of ice above the water jacket, as was done in the tests represented in Figure 4, the seeds germinated somewhat better if placed in the top of the cool chamber than if placed lower in the chamber where the temperature changes were less abrupt.

In the tests discussed in the two preceding paragraphs the temperatures in the positions in which the seeds germinated best were almost always somewhat lower than in the other parts of the chambers. The differences in temperature were not, however, commensurate with the corresponding differences in germination. In the more rapidly cooled and constantly somewhat cooler parts of the cool chambers convection currents of air must have been more lively than in other parts, and this may have led to a significantly better renewal of oxygen in immediate contact with the seeds, as suggested by Vanha (21). Also there was a constant tendency in the cooler parts of the chambers for water from the air which had become saturated in the warmer parts of the chambers to condense upon the surface of the seeds. This condensation water would presumably be saturated with oxygen, which would be immediately available for the use of the seeds unless kept out by seed coats relatively impermeable to oxygen. While there is no direct evidence that oxygen relations really are important here this possibility must be admitted.

BERMUDA GRASS

The favorable effect of temperature alternations is strikingly evident in Figure 5. A very narrow alternation (5° C.) is almost without effect, and a 10° alternation is much less effective than a 15° or 20° alternation.

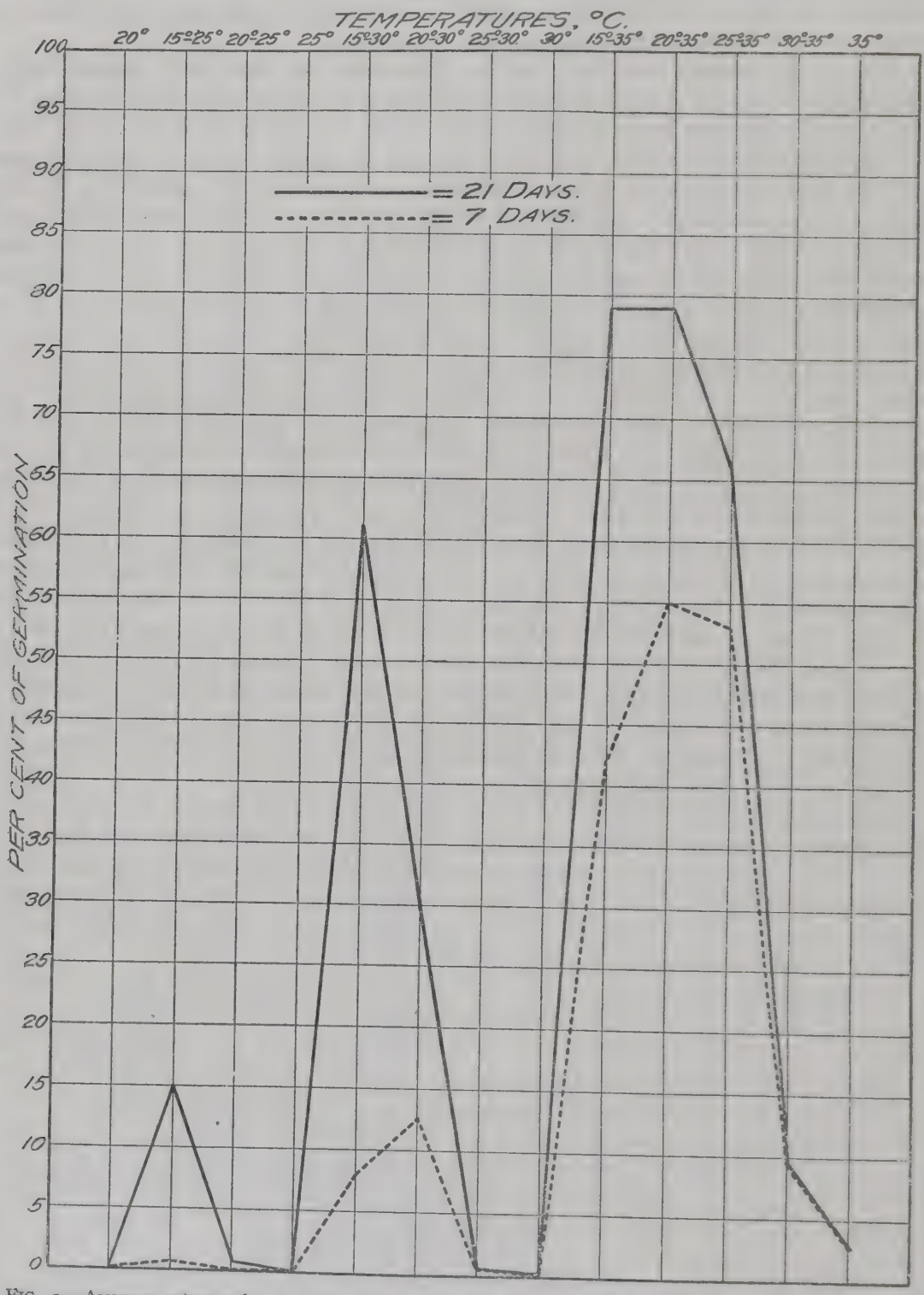

FIG. 5.—Average rates and percentages of germination of three lots of Bermuda grass seed under 13 temperature conditions.

Bermuda grass seed germinates at high temperature, its germination being better at 35° and with the 5° alternation below 35° than at lower constant temperatures or with 5° alternations in a lower temperature

range. In view of the temperature effects here manifested and the work with Johnson grass seed discussed later in this paper, it is unfortunate that experimental germination tests of Bermuda grass seed were not also made with still warmer temperatures, both constant and in alternation.

ORCHARD GRASS AND PARSNIP

These two kinds of seed required an alternation of temperatures for best germination. They were considerably more sensitive to temperature conditions, especially to the difference between constant temperatures and temperature alternations, than redtop seed (fig. 2.), but somewhat less sensitive than Kentucky bluegrass (fig. 3) or celery seed (fig. 4). With each of these two species every alternation of temperatures except 25° C. to 30° and 30° to 35° gave better results than any constant temperature.

Reverse Alternations

Parsnip and celery seed were tested with the alternations 25° C. for 16½ hours and 20° for 7½ hours each day in comparison with the alternation 20° for 16½ hours and 25° 7½ hours each day and with the similar alternations 30° to 20° and 20° to 30°. The alternations in which the cool temperature was maintained for the longer time gave uniformly better germination than the reverse of these alternations. The differences were frequently large, but with some lots of seed they were insignificant.

JOHNSON GRASS

It has already been pointed out (*11*): (1) That newly matured Johnson grass seed is characterized by a dormancy which is not resident in the embryo but is imposed by coat structures and which is not entirely removed until after several months of dry storage, and (2) that both during and after the process of after-ripening the seeds of this species germinate better at a high temperature and with temperature alternations than at lower or constant temperatures. In the earlier paper (*11*) an alternation between 25° and 40° C. was given as the optimum temperature condition for the germination of this kind of seed, but later work has shown that the still higher temperature alternation 30° to 45° gives still better results.

Figure 6 shows the average rates and percentages of germination of six lots of fully after-ripened Johnson grass seed under 12 temperature conditions, the higher temperature in each alternation being maintained from six to eight hours each day.

The accelerating effect of warm temperatures is shown by the fact that, in contrast to all the kinds of seed hitherto discussed, Johnson grass seed germinated more rapidly in the early days of the germination test at constant temperatures than with the temperature alternations corresponding to a lower mean temperature, but just about the same as with temperature alternations corresponding to the same mean temperature. The high points in the graph for germination in two days all correspond to constant temperatures, but the points in this graph showing germination with the temperature alternations 20° to 35° C., 25° to 40°, and 30° to 45°, fall on almost exactly the same levels, respectively, as those for the approximately equivalent constant temperatures 25°, 30°, and 35°.

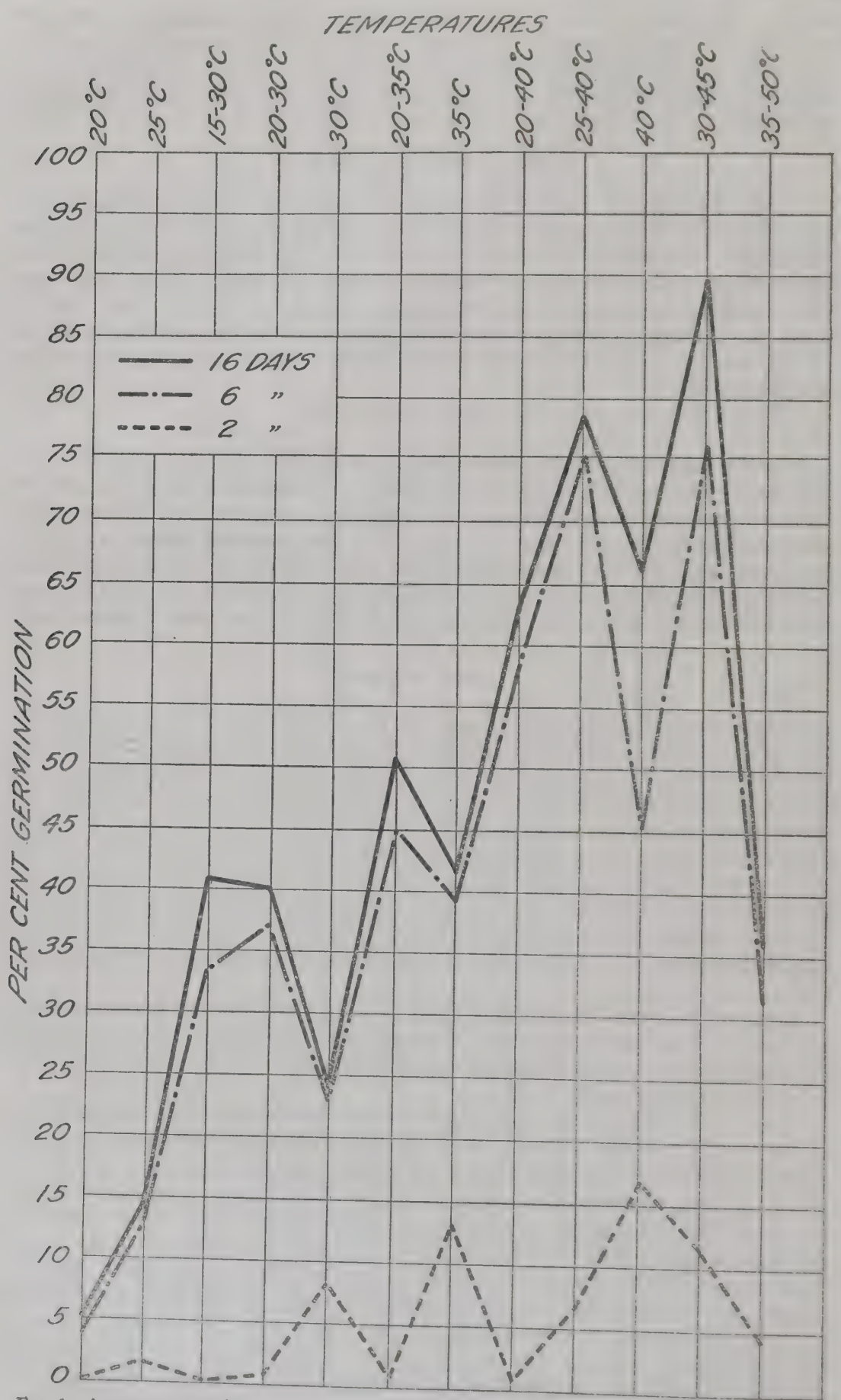

FIG. 6.—Average rates and percentages of germination of six lots of Johnson grass seed under 12 temperature conditions.

As germination advanced the alternations proved more advantageous and the shape of the graphs representing germination became reversed, with the low points falling on the lines for constant temperatures.

The alternation 30° to 45° C. gave best results, and 25° to 40° next best.[3] With the alternation 30° to 45° germination was more rapid at first, progressed at a more nearly uniform rate, and was more active in the last 10 days of the germination test than with the alternation 25° to 40°. With the latter alternation nearly all of the germination occurred between the second and the sixth days.

As with Kentucky bluegrass seed (see p. 301), the effect of the mean temperature of the alternations supplements in a definite manner the more important effect of the temperature changes as such. The alternation 20° to 30° C. evidently is not wide enough to produce the desired results. Table II compares the other alternations used and the percentages of germination on the basis of the approximate mean temperatures of the alternations. The percentage of germination rose regularly with rising mean temperature up to 34° and then fell abruptly.

At 40° C. constant, germination was spread over an even longer period than with the alternation 30° to 45°. It is important to remark here that while some seeds produced normal seedlings at this high temperature (40°) many at this temperature and a few at a constant temperature of 35° germinated only sufficiently to spread the heavy scales which inclose the caryopses by the slight elongation of radicle or coleoptile, after which growth ceased. These seeds were counted as germinated. When such seeds, after growth had stopped, were placed at a cooler temperature, either constantly or in alternations, normal growth ensued. One is reminded here of Kidd & West's work (*14*) in which a variety of agents induced growth in dormant white mustard seeds when used in concentrations or degrees just falling short of causing serious injury to the seed. According to this conception heat might here be considered as a stimulus to germination; and the advantage of an alternation between temperatures, the warmer of which lies either near the upper limit of endurance of a given seed or above the optimum for its germination and the subsequent growth of the seedling, might lie in the fact that the seed is given the temporary and recurring advantage of an elevation of temperature without being subjected to the harmful effects of long-continued exposure to a high temperature. In this connection it should be remembered also that in the case of a temperature alternation the actual temperature of the seeds probably never quite reached the temperature shown on the thermometer in the warmer germinating chamber and that the rise and fall in the temperatures of the seeds were relatively slow and gradual.

TABLE II.—*Temperature alternations, corresponding mean temperatures, and average percentages of germination produced in six lots of Johnson grass seed*

Temperature alternation.	Approximate mean temperature.	Average percentage of germination.	Temperature alternation.	Approximate mean temperature.	Average percentage of germination.
°C.	°C.		°C.	°C.	
15 to 30	19	41	25 to 40	29	78
20 to 35	24	51	30 to 45	34	88
20 to 40	25	63	35 to 50	39	36

[3] Auxiliary viability tests of the seeds remaining ungerminated with the alternation 30° to 45° C. were made by removing the scales and pricking according to a method previously described (*11*). They showed for the seeds originally put to germinate an average viability of 93 per cent, which is only 4 per cent greater than the germination without special treatment.

With the extremely warm temperature alternation, 35° to 50° C., the percentage of germination was low and the majority of the seedlings failed to make normal growth.

Concurrent germination tests of the six lots of seeds whose germination is represented in Figure 6 were made also at 15°, 45°, and 50° C. and with the temperature alternation 40° to 55°. Less than 1 per cent germinated at 15° C., and these made but little growth. At the higher temperatures none germinated in six days. These seeds were then given the temperature alternation 25° to 40° C. for seven days, followed by viability tests [4] with the alternation 30° to 45° C. Viability tests showed that all but a fraction of 1 per cent had been killed by six days' exposure to 50° C. or the alternation 40° to 55°. A temperature of 45° C. for six days had killed more than half of the seeds of some of the lots, but only a few of the seeds of other lots. The seedlings produced were relatively weak.

Differences between individual lots of seed

Different lots of Johnson grass seed vary greatly in the readiness with which they germinate, and it has been found that poorly germinating lots are more sensitive to temperature conditions than lots which germinate more readily. This is illustrated in Figure 7 which represents the total germination in 16 days of two of the lots included in the averages for Figure 6. No. 8599 was grown at Arlington Farm, Va., and gave a fairly ready germination. The San Antonio lot was collected from wild plants near San Antonio, Tex., and gave poor germination. While the general shape of the two graphs in Figure 7 is the same, favorable alternations and especially the best alternation, 30° to 45° C. showed greater advantage over constant temperatures and less favorable alternations in the case of the San Antonio lot than in the case of the other lot. Viability tests [5] showed 94 per cent of the San Antonio lot and 95 per cent of the other lot to be alive and capable of producing vigorous seedlings.

Effect of removal of the scales

The effect of removal of the scales (glumes, or hulls) upon the germination of Johnson grass caryopses has already been pointed out (*11*). The effect is illustrated in Figure 8, which shows the average percentage of germination in 13 days of two lots of seed intact and with the scales removed. The increase in germination as a result of removing the scales is especially noteworthy at the warm constant temperatures 30°, 35°, and 40° C.

In the preceeding section it was shown that readily germinating lots were less sensitive to temperature conditions than lots which germinated poorly. Increasing the readiness with which a given lot germinates and at the same time diminishing its sensitiveness to temperature conditions by removal of the scales merely constitutes a special case of the previous rule. The rapidity of germination also is greatly increased by removal of the scales.

[4] See footnote on page 307.
[5] See footnote on page 307.

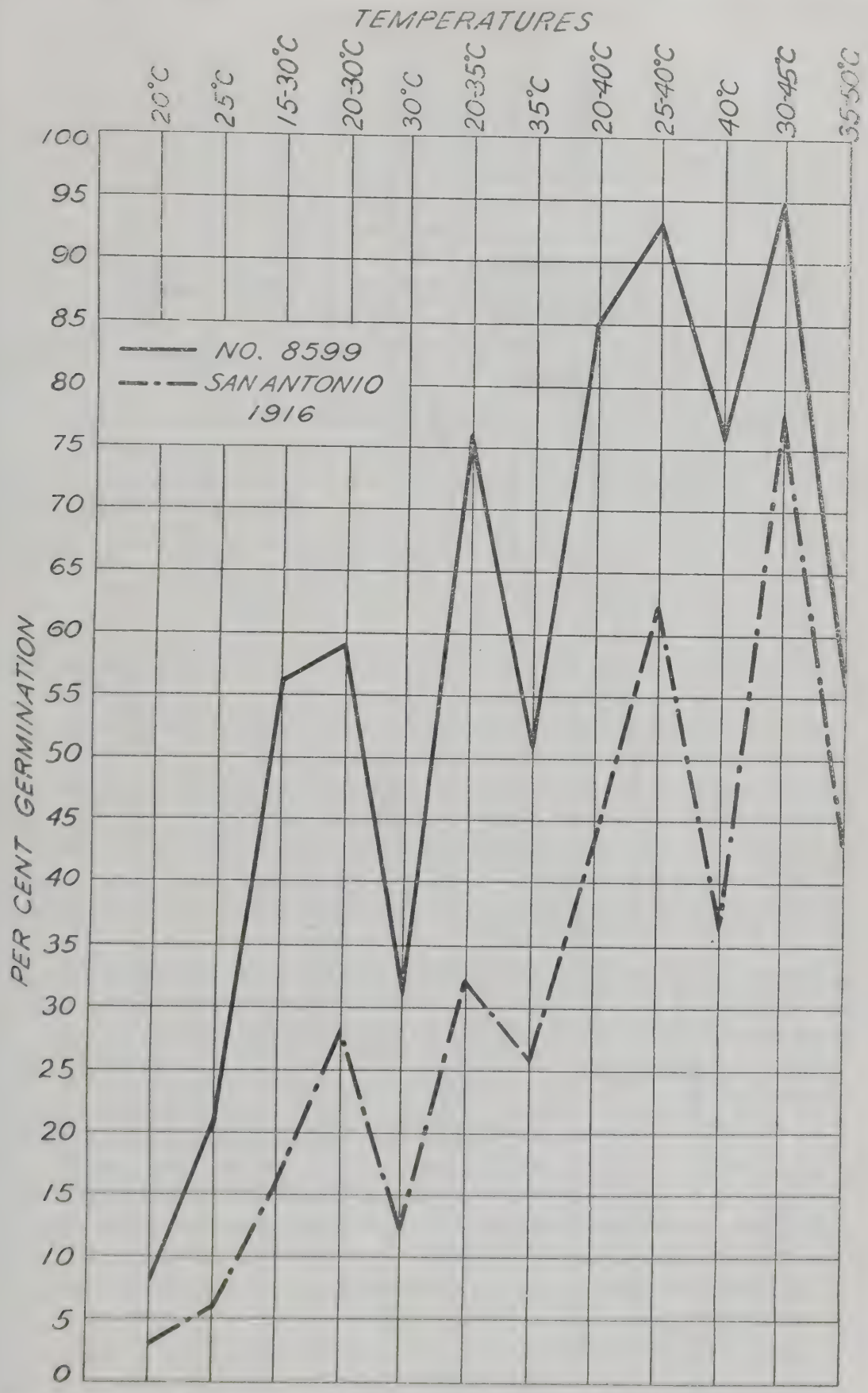

FIG. 7.—Percentages of germination in 16 days of two lots of Johnson grass seed under 12 temperature conditions.

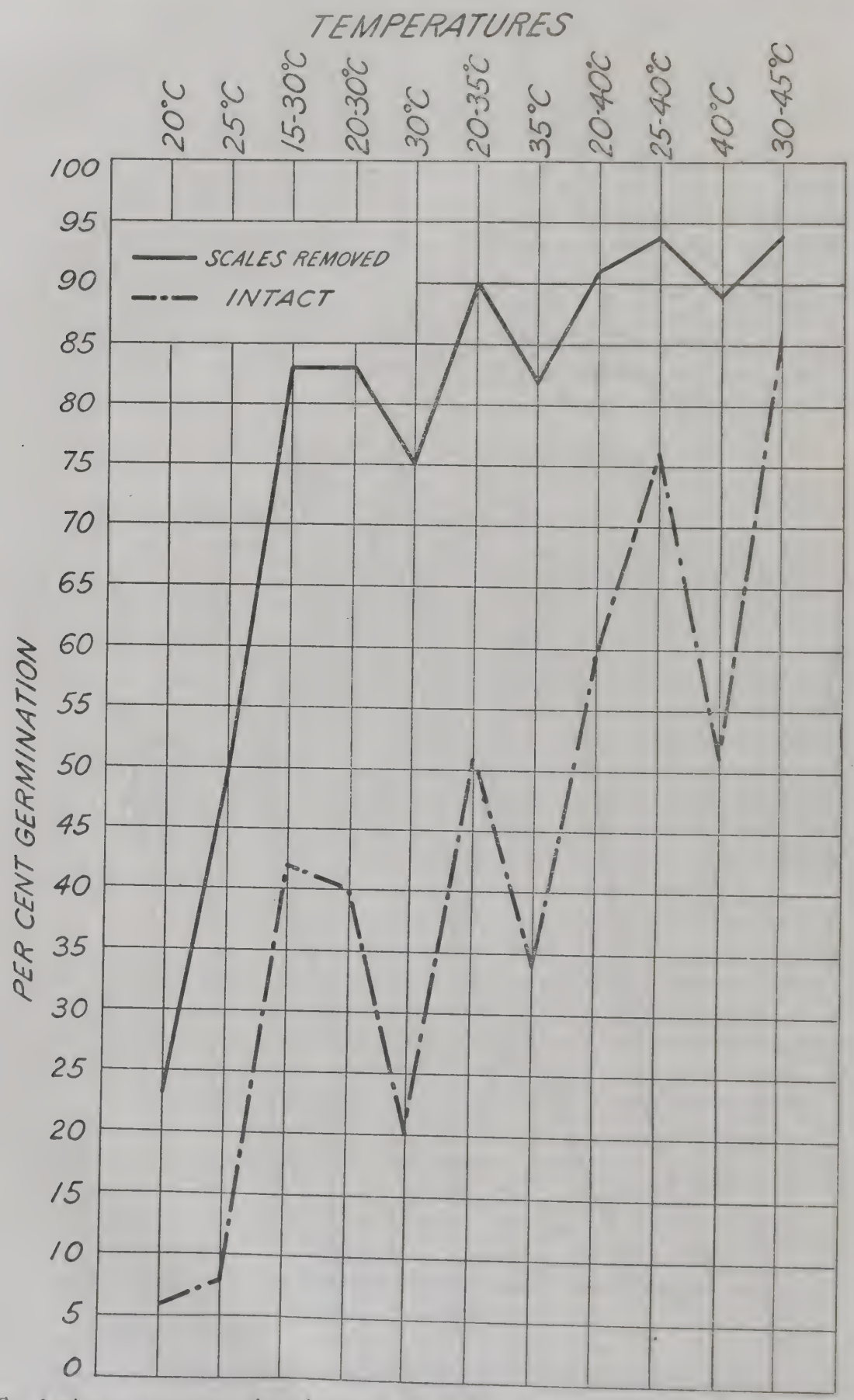

FIG. 8.—Average percentages of germination of two lots of Johnson grass seed, intact and with scales removed.

Effect of various lengths of time at the warmer temperature of an alternation

Six lots of seed were germinated with temperature alternations of 25° to 40° C. and 30° to 45° C., in which the seeds were in the warmer chamber two, four, six, and eight hours each day. Figure 9 shows the average germination. The numerals in the position of exponents to the temperature conditions at the top of the figure indicate the number of hours at the warmer temperatures. This figure illustrates again the fact noted in connection with Figure 6, that germination is more rapid during the first few days, proceeds at a more nearly uniform rate, continues for a longer time, and reaches a higher total percentage with the alternations 30° to 45° than with the alternations 25° to 40°. Eight hours daily at 45°, however, reduced the rapidity of germination in the first two days below that with the alternations 25° to 40°. The rapidity of germination with the alternations 25° to 40° increased regularly as the length of time in the warmer germinating chambers increased.

Differences in response of individual lots of seeds to these eight temperature conditions are shown in Figure 10 which gives the percentages of germination of three lots of seed in 13 days.

No. 8604 was a readily germinating lot composed of seed about 10 months old. The germination of this lot was, within the limits of experimental error, identical under all eight temperature conditions.

No. 1417 was about 2 years old. It germinated much better with the alternations 30° to 45° C. than with the alternations 25° to 40°, but showed little difference according to the length of time in the warmer chamber.

The lot designated "San Antonio, 1917," was harvested from wild plants near San Antonio, Tex., a few weeks before the tests were made and had not after-ripened. In contrast to No. 1417, this lot germinated better with the alternations 25° to 40° C. than with those 30° to 45°; with both sets of alternations the percentage of germination decreased with increase in the length of time in the warmer chamber. At the end of 13 days this lot of seed was still germinating at the rate of about ½ per cent per day, more rapidly with the alternations 30° to 45° C. than with the alternations 25° to 40°, so that the germination with the two sets of alternations would have become more or less equalized with longer germination period. Whether the difference between this lot and No. 1417 is characteristic of differences between old seed and fresh seed not after-ripened is not known. Seed from the same locality as the San Antonio lot, collected one year earlier and tested at the same time, germinated better with all four of the alternations between 30° and 45° C. than with the alternations between 25° and 40°, in this respect resembling No. 1417. Furthermore, it is known that the germination temperatures of some seeds change with the progress of after-ripening. At any rate the differences obtained here illustrate the apparent impossibility of finding any single temperature condition which will be the optimum for every individual lot of seed, regardless of its peculiarities or its previous history.

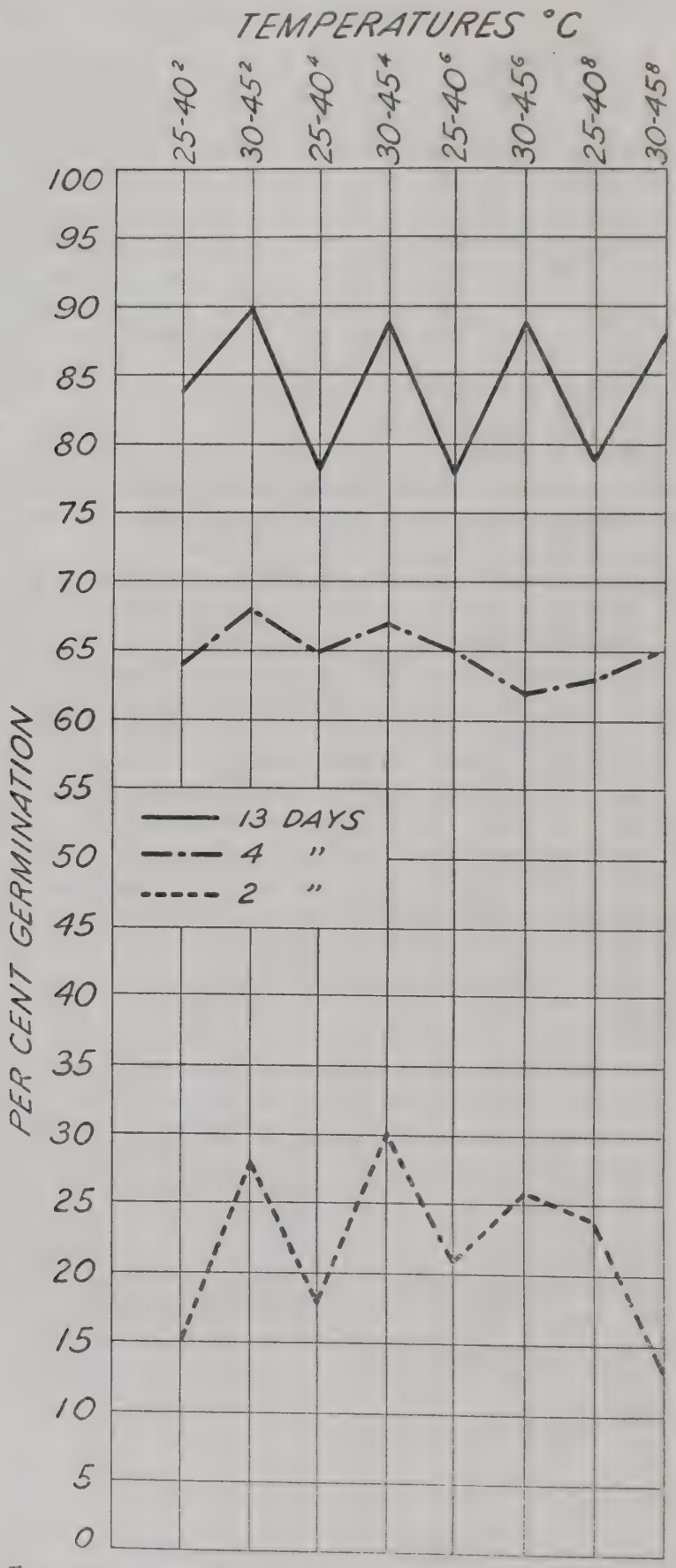

FIG. 9.—Percentages of germination of two after-ripened lots and one fresh lot of Johnson grass seed in 13 days with eight temperature alternations.

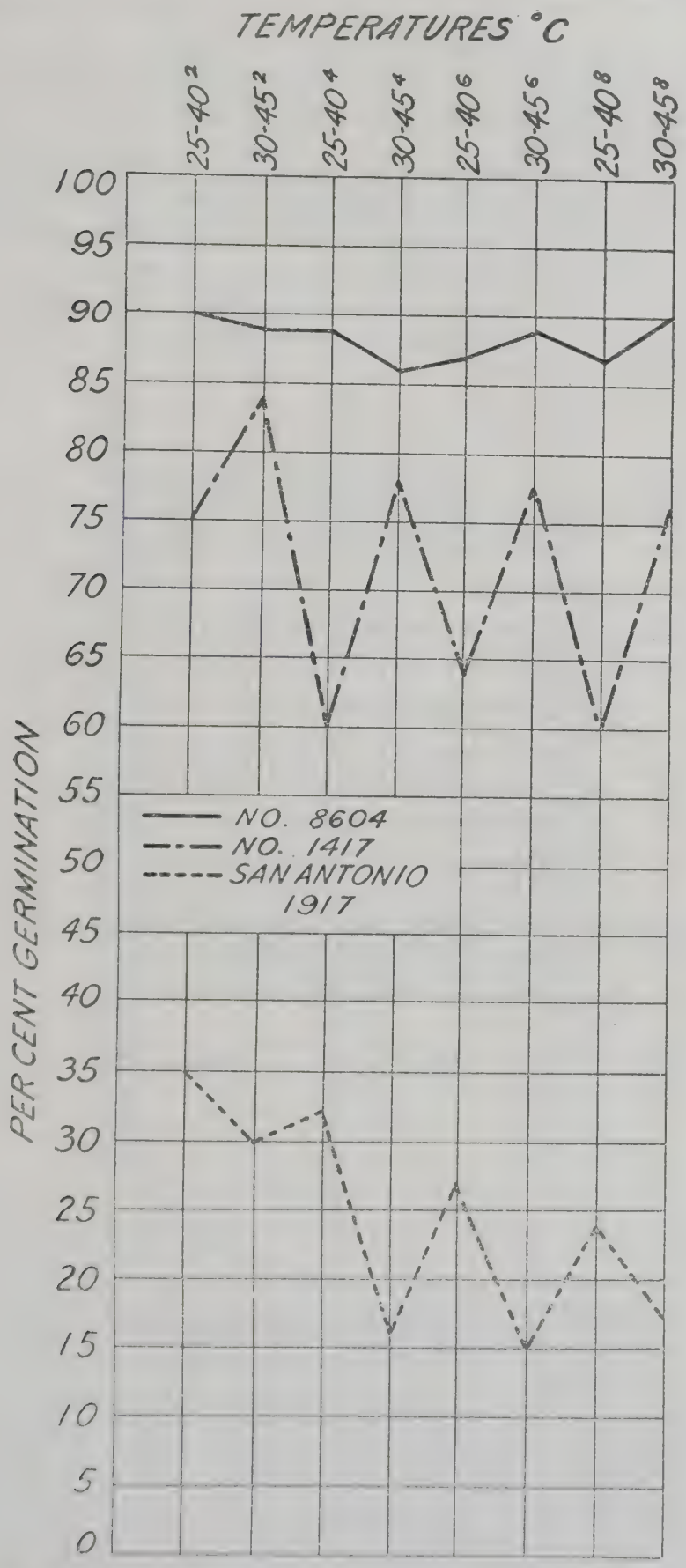

FIG. 10.—Average rates and percentages of germination of six lots of Johnson grass seed with alternations in which the seeds were in the warmer chambers for different lengths of time.

3. PROGRESS OF TEMPERATURE CHANGES IN RELATION TO GERMINATION: COMPARISON WITH FIELD TESTS

From December, 1911, to May, 1912, germination tests were made with about 30 kinds of seed, mentioned on p. 322, under 11 different temperature alternations.

Kentucky bluegrass seed was tested in the Jacobson apparatus (*4*); and the other kinds were placed between blotters or on top of blotters, according to the size of the seeds. Each chamber was constantly filled

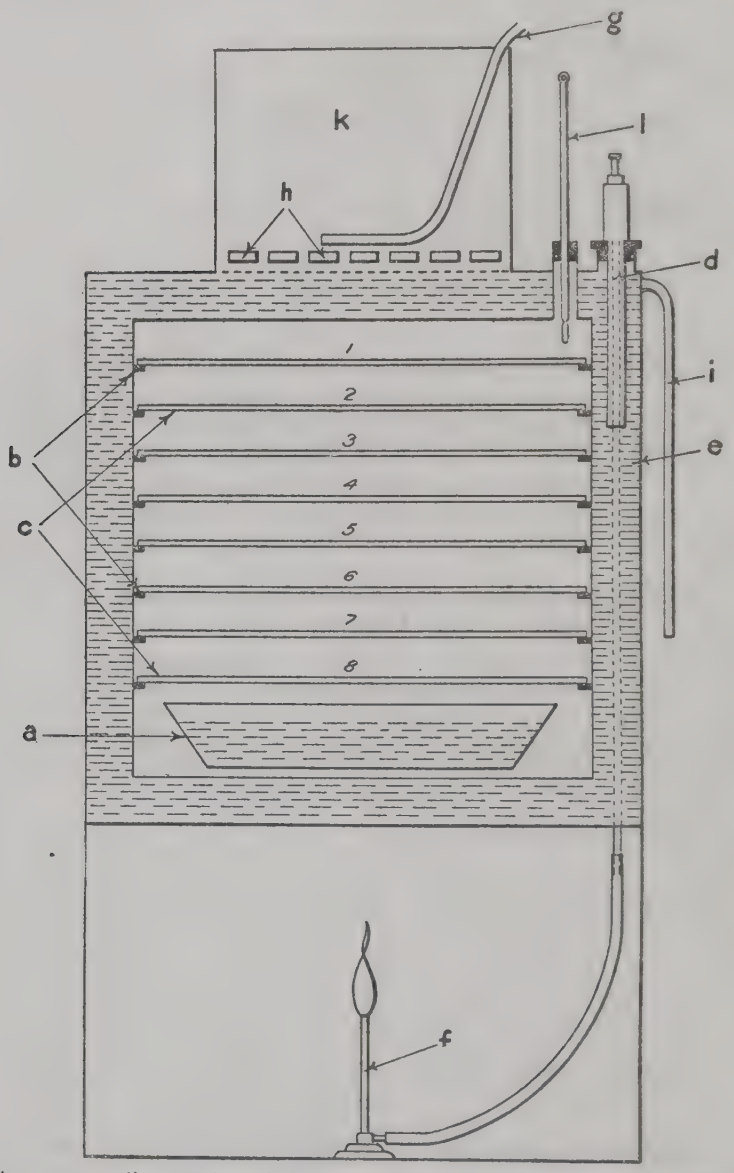

FIG. 11.—Median section of a germinating chamber: (*a*), pan of water; (*b*), ledges to support seed trays; (*c*), seed trays (numbered from 1 to 8); (*d*), thermo-regulator; (*e*) water jacket; (*f*), burner; (*g*), cold-water pipe; (*h*), perforated copper plate; (*i*) overflow pipe; (*k*), ice box; (*l*) thermometer.

to its capacity. At the same time a study was made of the progress of temperature changes in several different parts of the chambers. These temperature records were supplemented later by a more detailed study of temperature changes in a much larger number of positions in chambers filled with moistened blotters, as when the germination tests were in progress, but this time without the seeds. From these records time-temperature curves were constructed. Before considering these curves, and in order to make their significance clear, it is well to introduce a brief description of the germinating chambers.

GERMINATING CHAMBERS

All of the germination tests were made in copper chambers surrounded, except the door, by a water-jacket space, the water in this space being the medium of temperature control. Figure 11 shows one of the chambers in median vertical section.

The interior space of each germinating chamber is approximately a 20-inch cube. A pan of water (*a*) in the bottom of this space maintains a nearly saturated atmosphere in the chamber. At intervals of 2 inches on the sides of the chamber are copper ledges (*b*) for the reception of perforated copper trays (*c*) which carry the seeds and which in the figure

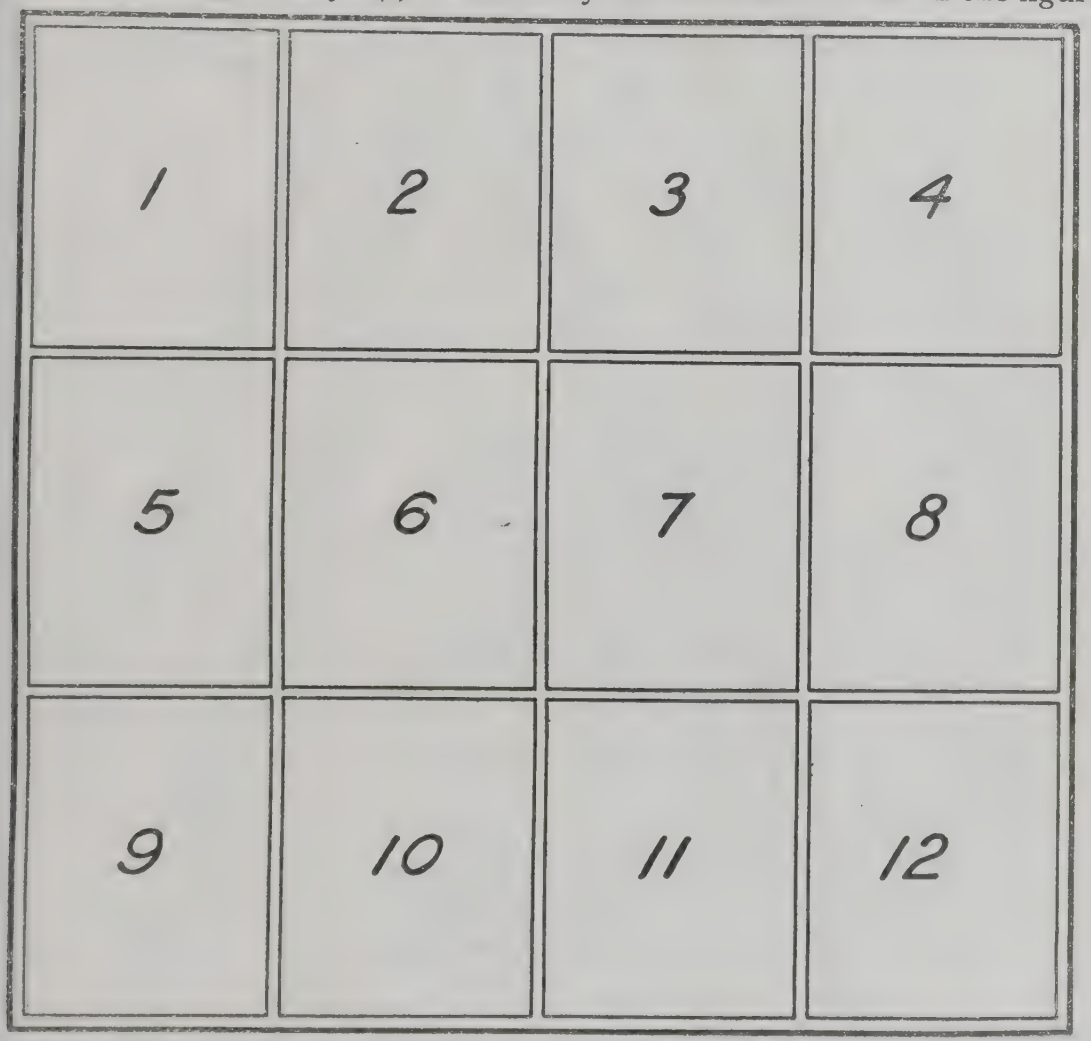

FIG. 12.—Diagram of a seed tray with folded blotters. The blotters are numbered from 1 to 12.

are numbered from 1 to 8, beginning at the top. The gas for heating the chamber passes through the thermo-regulator (*d*), which is inserted in the water jacket (*e*) and thence to the burner (*f*). The water for cooling the chamber enters the water jacket through the rubber tube (*g*) and the perforated copper sheet (*h*). The overflow passes out through the waste pipe (*i*). Whenever necessary, ice was placed in the ice box (*k*) and the water passed over it before entering the water jacket. The thermometer (*l*) indicates the temperature of the air in the extreme top of the chamber.

Each seed tray except No. 8, on which bluegrass seed was tested with the use of the Jacobson apparatus, received 12 folded blotters (4 thicknesses) for the small seeds, which were tested on top of blotters, and 24 (8 thicknesses) for the larger seeds, which were tested between blotters in duplicates placed on top of each other. Figure 12 shows the arrangement of the blotters upon a seed tray.

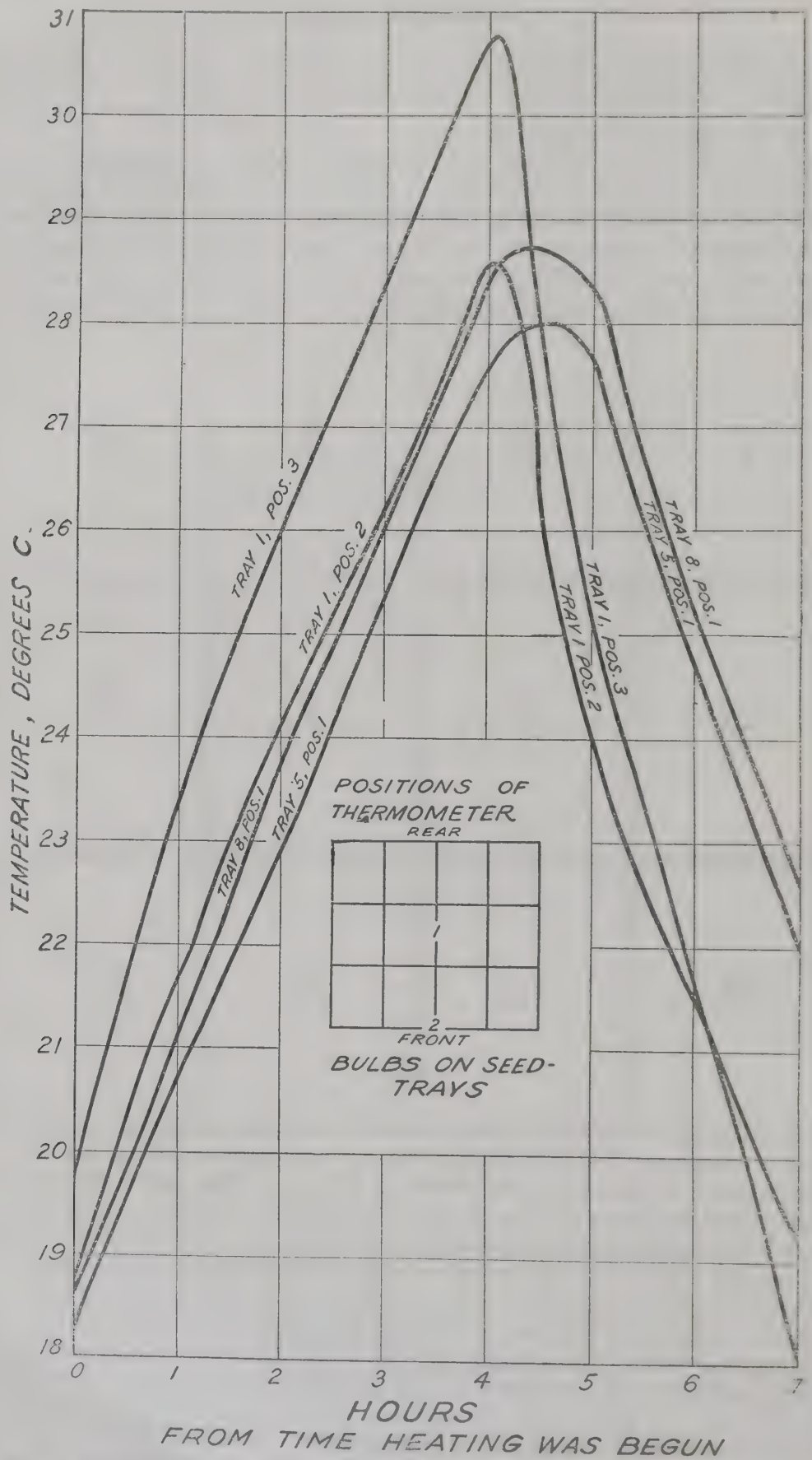

FIG. 13.—Curves showing changes of temperature on top of wet blotters in different parts of a germinating chamber which was heated by a gas flame below for four hours and then rapidly cooled by ice and cold water above for three hours.

TEMPERATURE CHANGES WITHIN THE GERMINATING CHAMBERS

As was anticipated, the temperature changes varied considerably in different parts of any given chamber. The heat imparted to the wet blotters and the seeds passed inward from the copper walls of the chamber; in cooling them the radiation was in the opposite direction. Naturally, the temperature changed more rapidly and therefore attained

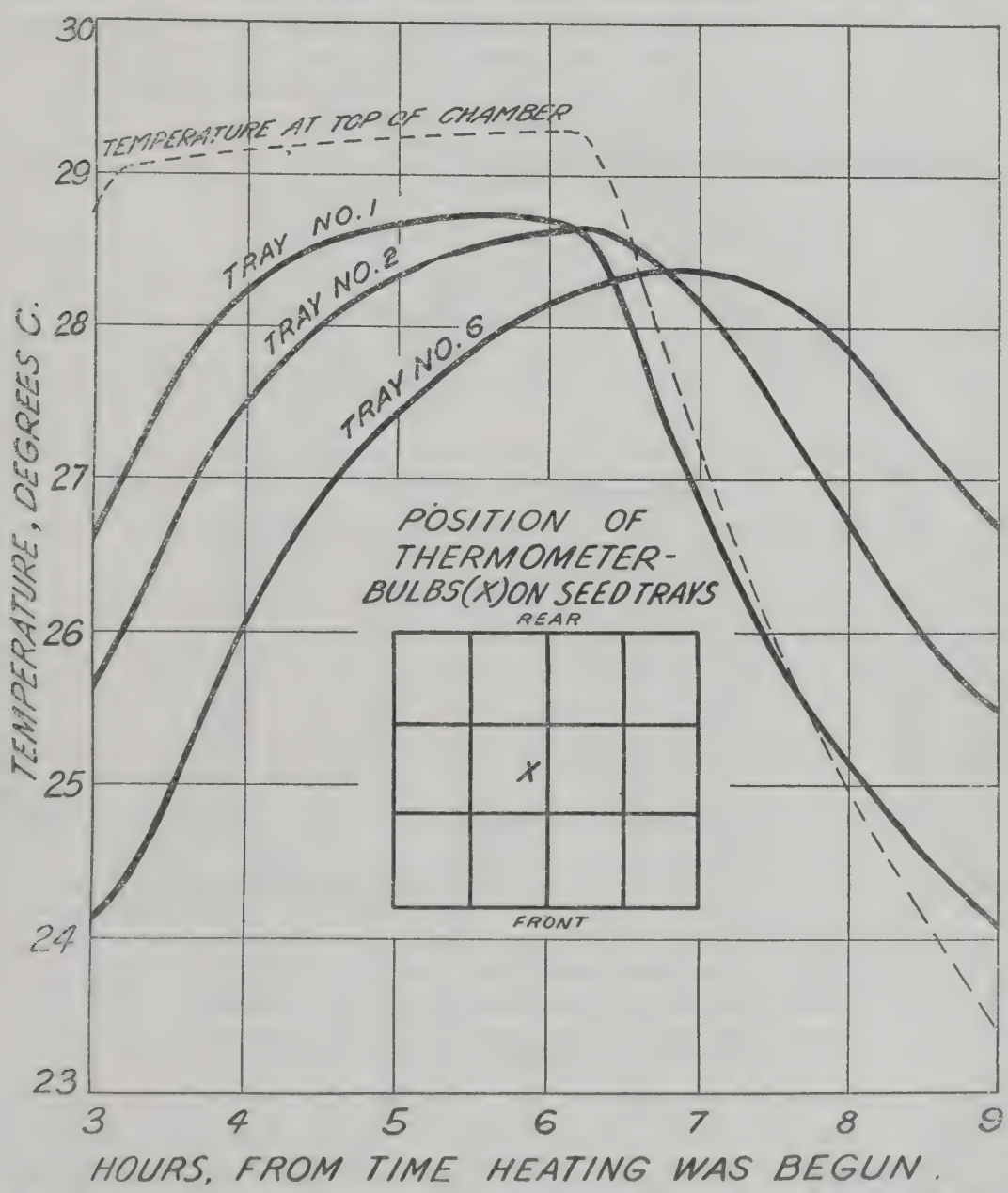

FIG. 14.—Curves showing changes of temperature within wet blotters in a germinating chamber which was heated three hours, then held at a nearly constant temperature for three hours, and finally cooled during three hours.

wider extremes near the top, bottom, and side walls of the chamber than near the center of the chamber. Furthermore, the rates of heating and cooling, especially of the seeds in the central parts of a chamber, were more rapid when only four thicknesses of blotting paper were used on each tray than when eight thicknesses were used, and were more rapid in the air between the seed trays than within the blotters themselves. These differences are illustrated in Figures 13, 14, 15, and 16.

Figure 13 shows the temperature changes as read from thermometers whose bulbs rested on top of four thicknesses of wet blotting paper in four different positions in a chamber which was heated by a gas flame below for four hours and then immediately cooled for three hours by means of ice and water above. The eight trays were completely covered with four thicknesses of folded blotters.

The curve for position 3 on tray 1 (near three walls of the water jacket) represents the most rapid and that for position 1 on tray 5 (near the center of the chamber) very nearly the least rapid temperature

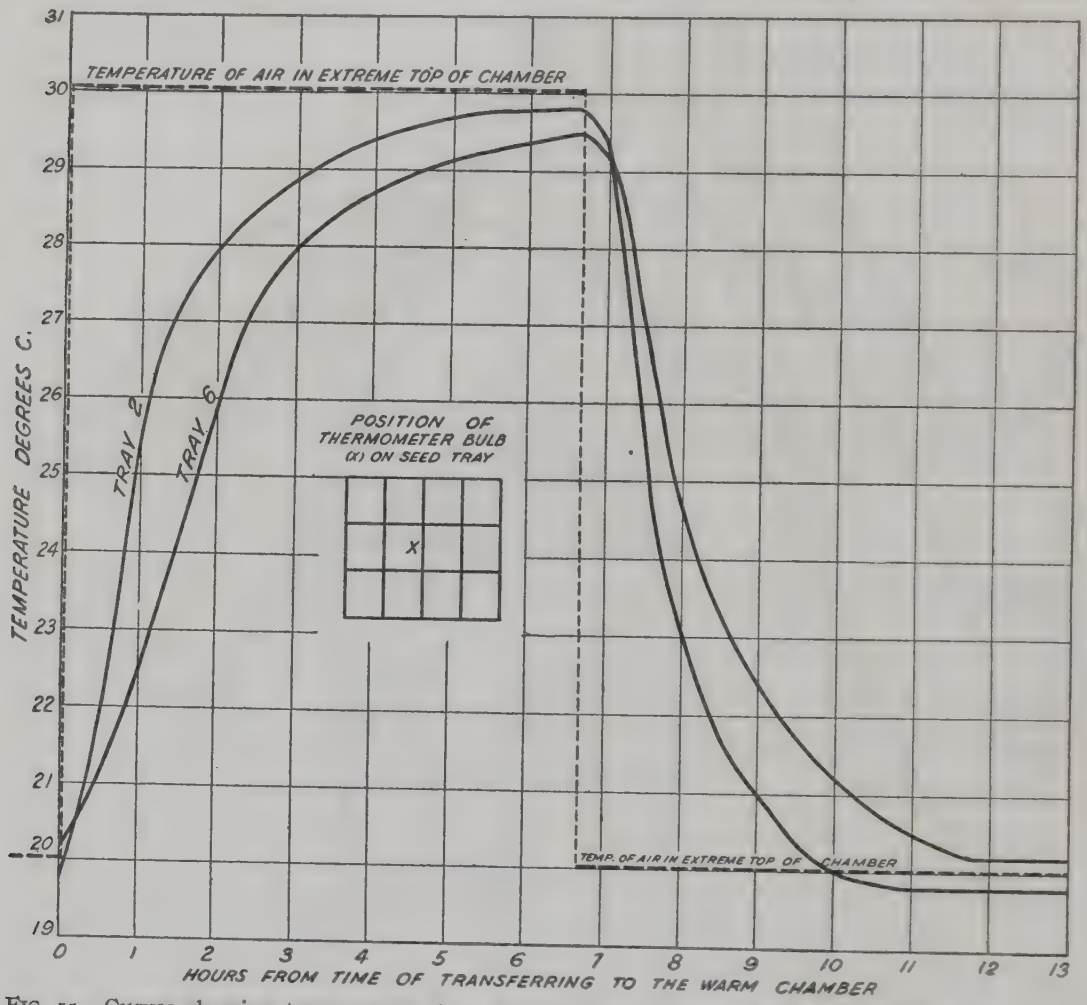

FIG. 15.—Curves showing temperature changes within wet blotters when these were transferred two germinating chambers at fixed temperatures.

changes occurring in any part of the chamber, the curve for position 2 on tray 1 (near the middle of the insulated door) represents the least rapid temperature changes for that tray; and the curve for position 1 on tray 8 (directly over the middle of the pan of water) represents a relatively slow rate of heating and cooling, combined with a period longer than in any other part of the chamber, during which the temperature was nearly constant in the neighborhood of the maximum temperature for that position.

The extremes of temperature in different parts of the chamber were 1.6° C. apart before the heating was begun, 3.2° when heating was discontinued, and 4.5° at the end of the 7-hour period. With the discontinuance of rapid cooling at the end of 7 hours, of course the temperature in different parts of the chamber became gradually equalized so that the temperature curves would converge if continued beyond the 7-hour period.

For a number of alternations the temperature of a chamber, as indicated by the thermometer in its top, was held at about its highest point for some time before cooling was begun. In these cases, as shown in

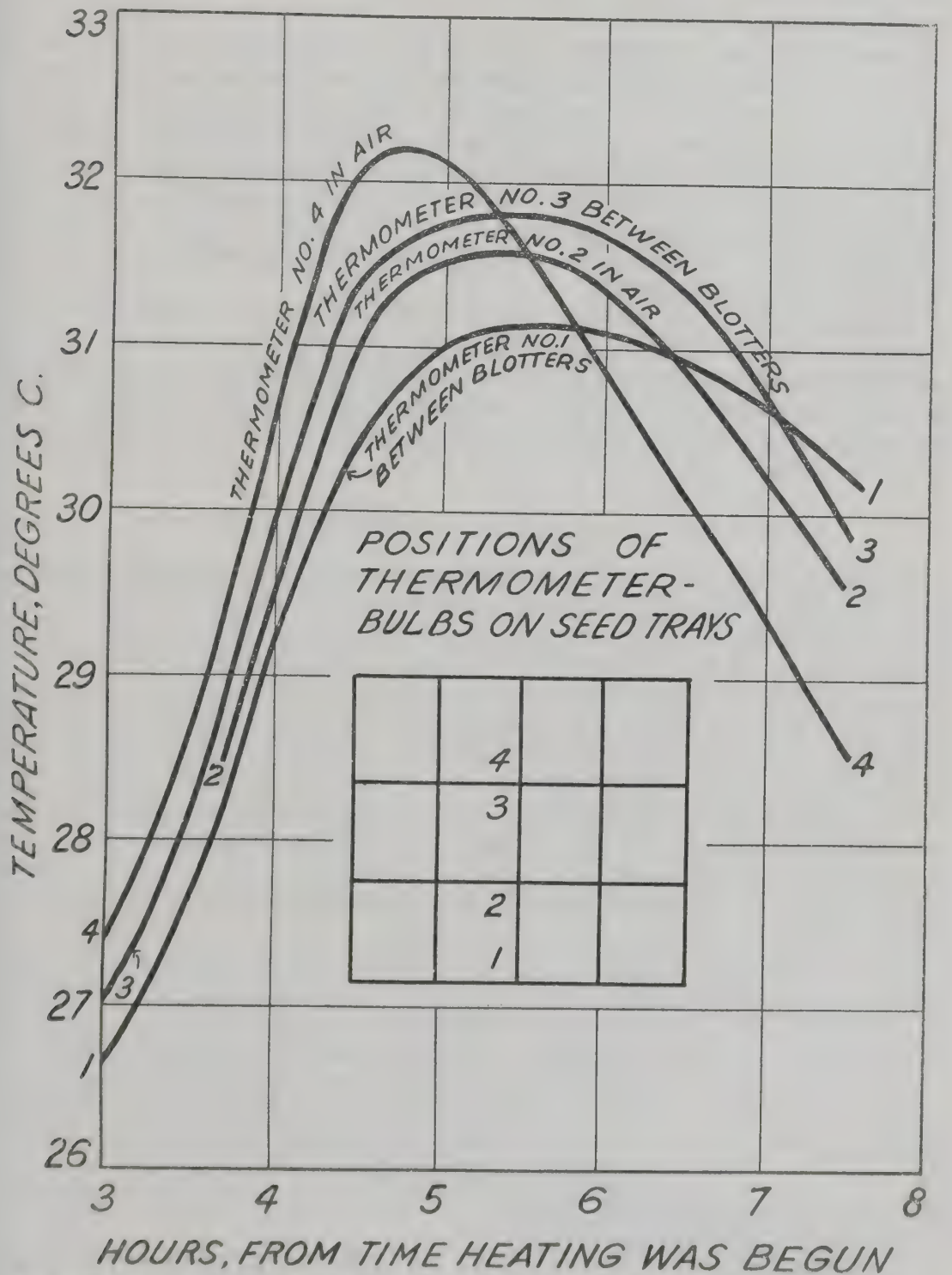

FIG. 16.—Curves showing temperature changes in four positions on top tray of a chamber which was heated and cooled. Thermometer bulbs No. 1 and 3 were within wet blotters; No. 2 and 4 were in the air about one-eighth inch above wet blotters.

Figure 14, the curves representing temperature changes within the chambers became broad and round at the top, instead of sharp and narrow as in Figure 13, and the highest temperature reached within the blotters was nearly the same in all parts of the chamber. Figure 14

shows the temperature for a number of hours within folded blotters near the middle of trays 1, 2, and 6, and also the temperature indicated by the thermometer in the top of the chamber. The effect is that of broadening the curves and displacing them to the left as viewed downward from the top tray.

Three of the eleven alternations were secured by transferring the seed trays with the seeds upon them between two germination chambers constantly maintained at fixed temperatures. In this case the temperature changes taken altogether were more nearly identical in different parts of the chambers, and approximate equilibrium in temperature between the different parts of the chambers was assumed much more quickly than in the two types of alternations previously discussed. Figure 15 shows the temperature changes within wet blotters near the middle of trays 2 and 6 in such an alternation.

Figure 16 shows much more rapid cooling of the air in the top of a chamber which is being cooled by ice and cold water above than within the blotters on the seed tray just below this layer of air. Such differences as here shown might be significant in determining whether, in a given case, a given kind of seed, for instance celery, will germinate better between blotters or on top of blotters.

COMPARISON OF THE DIFFERENT TEMPERATURE ALTERNATIONS

While the differences in the progress of temperature changes between different parts of a given chamber were frequently large, they were always less than the differences in the same part of different chambers which were used for different alternations. For the purpose of comparing the temperature changes in the different alternations, a position near the center of tray 6 was selected. Figure 17 shows the time-temperature curves for this position in all 11 alternations, which are numbered from 1 to 11 for convenience. The curves were drawn from actual observations for the first 15 hours and were projected for the remaining 9 hours of the day. Since the initial temperature varied in each case a few tenths of 1° C. from day to day but was always very near 20°, the curves have been slightly rectified so as to begin each at exactly 20°.

Alternations No. 1 to 3 were obtained by transferring between two chambers at fixed temperatures; No. 4 to 6, 8, and 10 by heating and then immediately cooling a single chamber; No. 7 and 9, by heating a chamber and then holding it for three hours at the highest temperature reached before beginning to cool it; No. 11 was obtained by heating a chamber and then allowing the temperature to fall naturally, without artificial cooling. The rates of heating and cooling were approximately the same from day to day in any one alternation. In all alternations after the temperature had fallen to 20° C. it was held nearly constant until heating was begun the next day.

The alternations differed widely in maximum temperature attained, mean daily temperature, and rate of cooling; but the heating was relatively rapid in all cases and the minimum temperature was the same in all cases.

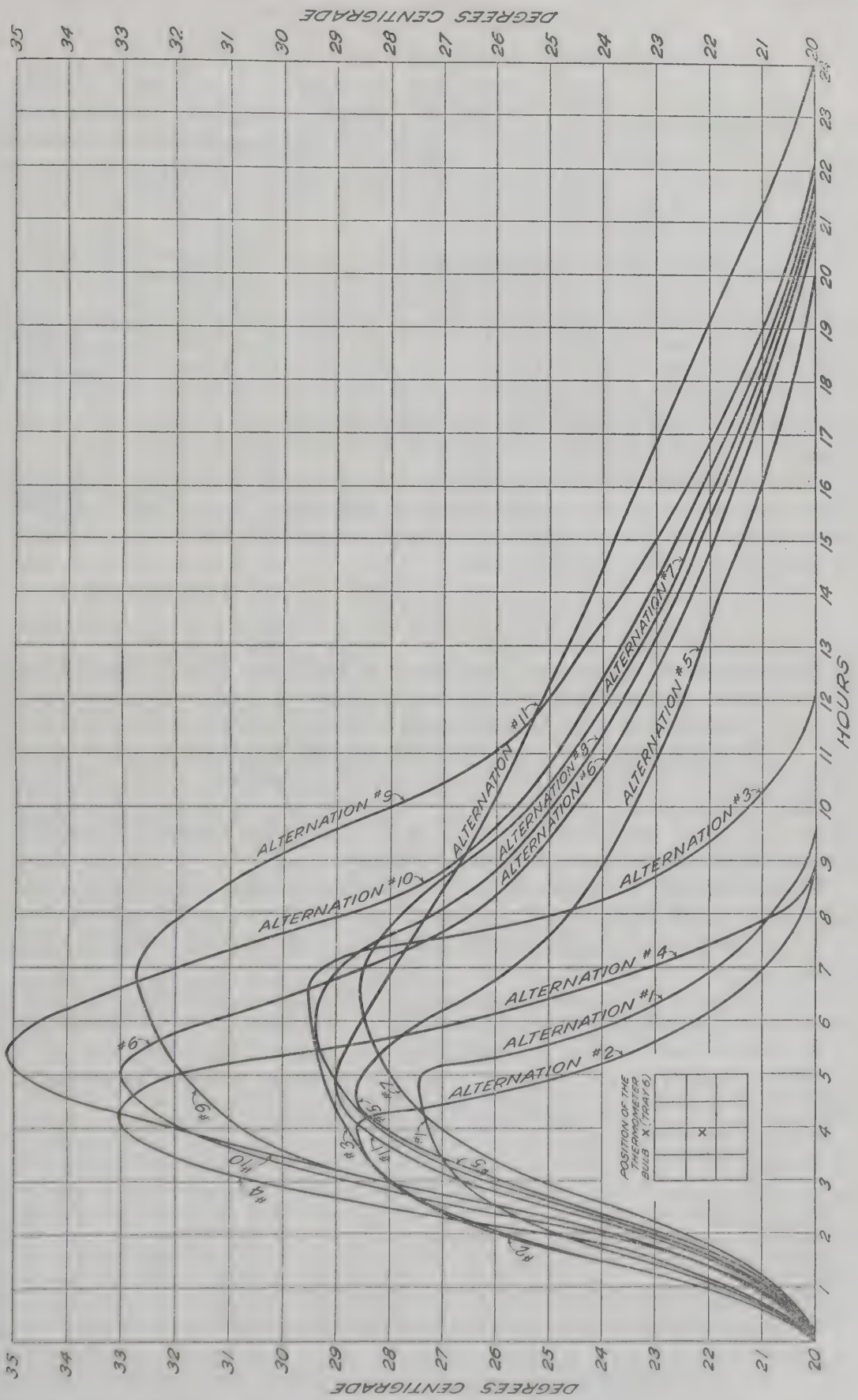
DEGREES CENTIGRADE
35 34 33 32 31 30 29 28 27 26 25 24 23 22 21 20
HOURS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
ALTERNATION #11
ALTERNATION #7
ALTERNATION #5
ALTERNATION #3
ALTERNATION #6
ALTERNATION #9
ALTERNATION #10
ALTERNATION #4
ALTERNATION #1
ALTERNATION #2
POSITION OF THE THERMOMETER BULB X (TRAY 6)

FIG. 17.—Curves showing temperature changes near the middle of tray No. 6 in 11 alternations of temperature.

GERMINATION WITH THE DIFFERENT ALTERNATIONS

1. Seeds which germinated equally well with all alternations

Seeds of carrot, cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), millet (*Chaetochloa italica* (L.) Scribn.), onion (*Allium cepa* L.), parsley, redtop, sorghum (*Holcus sorghum* L.), timothy, and watermelon (*Citrullus vulgaris* Schrad.) germinated with equal completeness with all of the temperature alternations from No. 2 to No. 11. They were not tested with alternation No. 1.

Seeds of bean (*Phaseolus vulgaris* L.), beet (*Beta vulgaris* L.), upland cotton (*Gossypium hirsutum* L.), cowpea (*Vigna sinensis* (Torner) Savi), flat pea (*Lathyrus sativus* L.), flax (*Linum usitatissimun* L.), hemp (*Cannabis sativus* L.), meadow fescue, milo (*Holcus sorghum* L.), orchard grass, pea (*Pisum sativum* L.), rice (*Oryza sativa* L.), perennial rye grass, soy bean (*Soja max* (L.) Piper), black bitter vetch (*Vicia ervilia* (L.) Willd.) were tested only with alternations Nos. 3, 5, 6, 7, and 9 but germinated with equal completeness with all of these alternations.

Some of the kinds of seed mentioned in the two preceding paragraphs have been shown in the earlier pages of this paper to germinate equally well and others almost as well at constant temperatures as with any alternation (see fig. 1, and p. 297).

2. Seeds which did not germinate equally well with all alternations

This class includes only three of the kinds of seed used in this part of the investigation; celery and bluegrass, which have already been shown strictly to require alternations of temperature for their best germination (see fig. 3 and 4 and text p. 299), and tomato (*Lycopersicum esculentum* Mill.). Table III shows the average percentages of germination of several lots of these three kinds of seed, together with a summary of the important features of each alternation, as shown by the time temperature curves of Figure 17. The germination period was 10 days for tomato, 26 days for Kentucky bluegrass, and 27 days for celery.

The percentage of germination of each kind of seed showed no definite correlation with the extreme or mean temperatures of the alternations or with the shape (whether narrow and abrupt or broad and rounded) of the upper part of the curves in Figure 17. There was, however, a definite relation between percentages of germination and rate of cooling, as shown by the temperature at the end of 15 hours, the number of hours of the day during which the temperature recorded was below 22.5° C., and the number of hours at 20° — changes shown by the abrupt or gradual fall of the right arm of the corresponding curves in Figure 17.

It must be admitted that the curves in Figure 17, which represent temperatures within blotters near the middle of the chambers, do not accurately represent the temperature changes occurring on top of blotters in the positions in which celery and bluegrass seeds were tested. It has already been shown, however (see fig. 13 and 17 and text, p. 320), that temperature differences in any one chamber in a given alternation were less than the characteristic differences between the different alternations. In all these tests the chambers were equally filled and each sample occupied the same position within the chamber in each test. Irregularities due to varying positions are therefore eliminated, and the conclusion is justified that an elevated temperature should not be long maintained. This agrees with the results of the reverse alternations discussed on page 305.

TABLE III.—*Germination of celery, Kentucky bluegrass, and tomato seed with 11 temperature alternations*

Alternation number.	1	2	3	4	5	6	7	8	9	10	11
Approximate maximum temperature (° C.)	27. 5	28. 5	29. 5	33. 0	28. 5	33. 0	28. 5	29. 5	32. 5	. 023	59. 0
Approximate mean temperature (° C.)	21. 5	21. 5	22. 5	22. 0	22. 5	24. 0	23. 5	23. 5	25. 5	24. 5	24. 5
Temperature at end of 15 hours (° C.)	20	20	20	20	21. 5	22. 0	22. 3	22. 2	23. 0	22. 7	23. 8
Number of hours below 22.5° C.	19	19	16	18	14	12	11	11	10	10	8
Number of hours at 20° C.	15	15	12	15	4	3	3	3	2	2	(*a*)
Average percentage of germination. Celery:											
5 lots of good seed	....	78	76	78	74	73	72	66	67	67	43
3 lots of poor seed	....	39	33	35	36	35	27	24	20	26	16
Kentucky blue grass:											
7 lots	65	68	64	64	63	61	59	58	60	59	50
Tomato:											
4 lots	....	....	73	....	71	68	66	....	59	....	....

[a] The temperature in this alternation sometimes did not fall to 20° C. in the entire 24 hours.

For best germination of the three kinds of seed here considered the temperature after its initial rise to a higher degree should fall at least as low as 21.5° C. within 15 hours from the time the heating was begun, should be below 22.5° for at least 12 hours of the day, and should be as low as 20° for several hours each day. These results could be attained by the method of transferring between two germinating chambers at fixed temperatures even if the seeds were kept as long as 10 hours in the warmer chamber. While such alternations were not used in this investigation the indications, both from the work on tomato seed recorded in this paper (Table III) and from Vanha's work (*21*), are that the longer period at the higher temperature would be injurious.

Altogether the investigation revealed an unexpectedly large degree of tolerance to a wide range of conditions in temperature alternations on the part of celery and bluegrass seed.

The method of transferring between two germinating chambers at fixed temperatures is much simpler to operate than heating and cooling a single chamber, on account of the difficulties in the way of accurate temperature control in the latter case; it involves more uniform conditions throughout an entire chamber and from day to day, and it gives at least as good results. It is therefore decidedly preferable to the other method.

3. Rapidity of germination

Celery and bluegrass seed germinated most rapidly with those alternations of temperature which also gave most complete germination and least rapidly with those alternations which gave least complete germination. Here, as in the earlier comparisons between alternations and constant temperatures (see p. 301), the special effect of a favorable alternation of temperatures predominates over the effect of low or high mean temperatures. This is in marked contrast to the temperature responses of constant-temperature germinators.

Parsley seed germinated more rapidly with alternation No. 5 than with a warmer alternation or with either more rapid or less rapid cooling.

All other kinds of seed germinated more rapidly with the warm than with the cool alternations. The differences were related to the rate and extent of cooling, the mean temperature, and the proportion of each day at a comparatively warm temperature, and were not related to the maximum temperature occurring in the alternation. Usually germination

was most rapid with alternation No. 9 and least rapid with alternation No. 4, although the upper temperature extreme was practically the same in the two alternations.

The germination of the seeds of the cucurbits was especially accelerated by the warm alternations. The average percentage of germination of watermelon seed in three days was 66 with alternation No. 9 and only 6 with alternation No. 4.

4. Uniformity of results with favorable alternations

Some lots of seed are much more sensitive to temperature conditions than other lots of the same kind. Consequently, while the most favorable temperature conditions give with every lot approximately the maximum germination attainable, less favorable conditions (for general use) may give equally good results with some lots and very poor results with others. A really very good, but temperature-sensitive, lot of seed may thus under slightly unfavorable temperature conditions germinate more poorly than a very poor lot which is not markedly sensitive to temperature conditions. It is impossible to say just what constitutes this different temperature sensitiveness of different lots of the same kind of seed, but it frequently becomes evident. It was strikingly exemplified by the celery seed used in this investigation. This suggests the possibility that some of the kinds of seed which in this investigation germinated as well with constant as with alternating temperatures may, under some circumstances—for instance, incomplete after-ripening—germinate better with an alternation.

Figure 18 shows the germination of eight lots of celery seed with four temperature alternations Nos. 2, 3, and 4, which were very favorable alternations, and No. 11, which was unfavorable. The curve for alternation No. 5 would follow almost exactly the course of that for alternation No. 2. Celery seed No. 87602, though one of the best germinators under favorable conditions, germinated less than half as much as the poorest lot when tested with alternation No. 11.

The difference in sensitiveness of different lots to temperature conditions is even more strikingly shown in Figure 19, which shows the germination of three lots of celery seed with 10 temperature alternations.

COMPARISON WITH THE RESULT OF FIELD TESTS[6]

Temperature changes in the soil during the spring and early summer

Several years ago, in connection with a series of field tests of various kinds of seed, records were kept of the temperature of the soil at various depths on Potomac Flats, near Washington, D. C. The period covered by the tests was from March 26 to June 28, inclusive. Nearly every day during this period temperature observations were taken at intervals of from 15 to 30 minutes, beginning at 4.30 a. m. and continuing to about 10.30 p. m.

From March 26 to April 22 the temperature of the soil at a depth of 1 inch was always below 30° C. and on several different days did not not become as warm as 20°. The period from April 22 to May 1 was somewhat warmer, but the temperature of the soil at 1 inch was never above 31°. and on one day barely reached 20°. On 18 of the 28 days in May for which there are records the daily range was from 20° or cooler to 30° or warmer. The other 10 days for which there are records were cooler. From June 1 to 7 and from June 11 to 15 the

[6] The data for soil temperatures and seedling production used in this comparison are from unpublished notes furnished by Mr. E. Brown, botanist in charge of the seed-testing laboratories.

weather was very warm. The temperature of the soil for the rest of June was only slightly warmer than in May.

The temperature range for 24 hours at a depth of 1 inch was nearly always more than 10° C., averaged about 16°, and not infrequently amounted to as much as 20°. The smallest range on any day was 6° and the largest 23.5°.

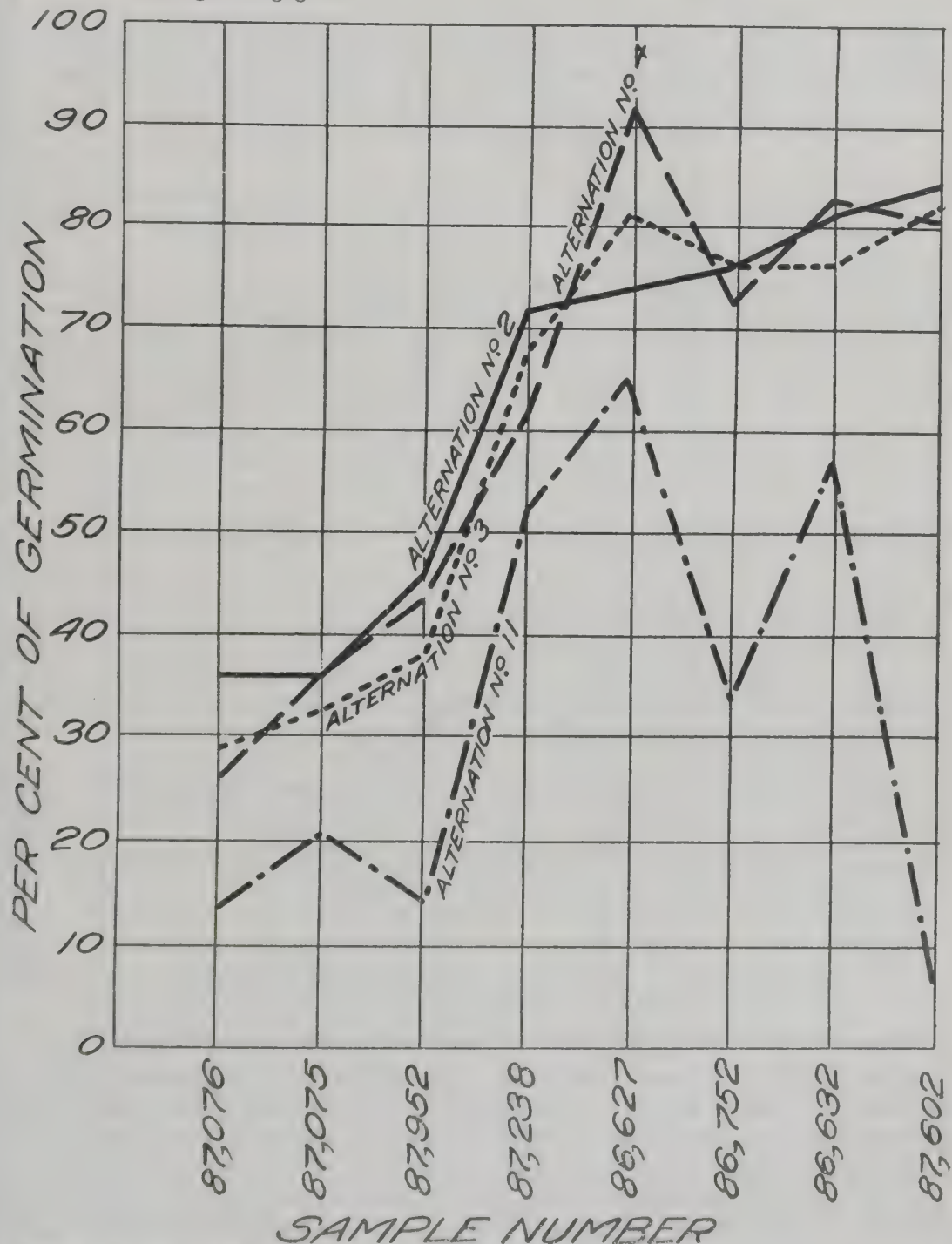

FIG. 18.—Percentages of germination of eight lots of celery seed with four alternations of temperature.

Seedling production in the field

Beginning with March 26, field tests were started every two or three days until early in June, seed from the same lots always being used. In each test 500 seeds of each lot were planted, and a record was kept of the number of days before the appearance of the first seedlings and of the number of seedlings appearing at intervals of a few days thereafter.

Of the seeds used in this field investigation, those of beans, beet, muskmelon, onion, parsley, peas, squash, tomato, and watermelon were included also in the chamber tests with different alternations of temperature previously discussed. It will be remembered that all of these except tomato germinated with equal completeness in all of the chamber tests but that the rapidity of germination varied according to the character of the alternation.

The bean, beet, onion, parsley, pea, and tomato seeds produced the first seedlings in the smallest number of days during the moderately warm

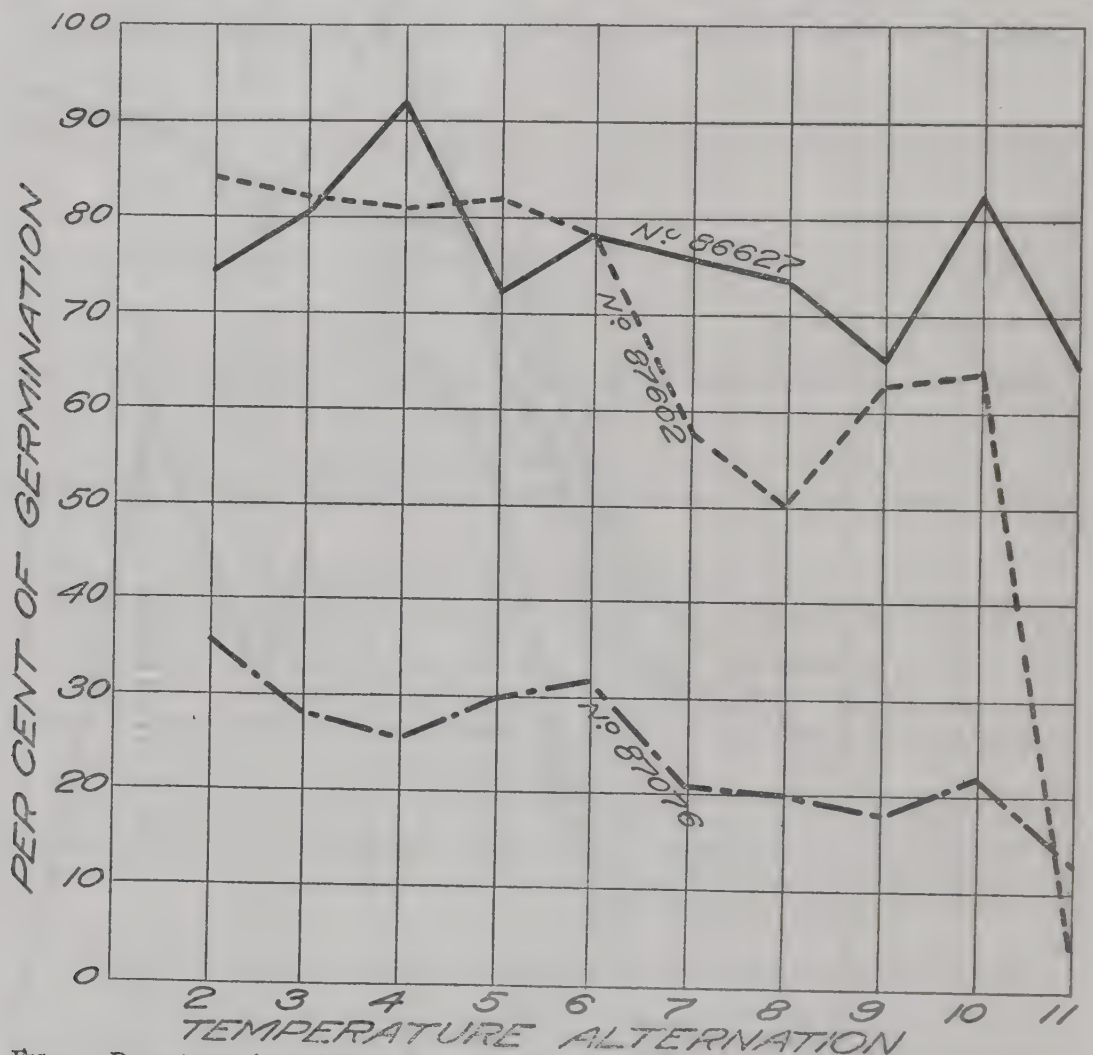

FIG. 19.—Percentages of germination of three lots of celery seed with 10 alternations of temperature.

weather of May or during the latter half of June, when the temperature was similar to that in May. Muskmelon, squash, and watermelon seeds produced the first seedlings in the smallest number of days during the very warm weather of the first half of June.

It should be remembered in this connection that the production of seedlings in the field involves not only the germination of the seeds but the piercing of the overlying soil by the young seedlings—a process which reflects the vigor of growth of the seedlings and may be retarded by excessively high temperatures, even when these same temperatures hasten germination as with Johnson grass seed (see p. 307). Since, however, the seedlings of the kinds of seeds here considered appeared normal and vigorous in all the alternations and also in the field, and since the chamber conditions giving most rapid germination gave also

most complete germination, the rapidity of germination in the chambers has been adopted as a basis for comparison with the promptness of seedling production in the field. The total percentage of seedling production is not considered here, because it is dependent upon other factors besides temperature conditions and does not allow of reliable comparisons in this connection.

COMPARISON OF SOIL TEMPERATURES WITH TEMPERATURES IN GERMINATING CHAMBERS

Figure 20 shows the time-temperature curves of three of the alternations which are represented in Figure 17, in comparison with those for the soil at the depth of 1 inch for four of the days during which the field tests were in progress.

The curves for April 16 and April 29 show the widest and the narrowest daily range in soil temperature occurring during the investigation. Both of these days came within a cool period of very slow seedling production. The curves for May 18 and June 27 fairly represent the soil temperature during the 2 periods—the larger part of the month of May and the latter part of June—during which the majority of the kinds of seed produced seedlings most promptly.

The soil temperature curves for May 18 and June 27 are similar in shape and range to those for chamber temperatures yet show some characteristic differences: (1) The lower temperature is well below 20° C. and the range is therefore greater; (2) the rate of cooling is less rapid than in case of the alternations which were secured by transfer between two chambers (No. 3) and much more rapid after about 5 p. m. than in the case of the alternations which were secured by heating and cooling a single chamber (Nos. 6 and 9), except in the case of No. 4 (fig. 17). Both of these soil temperature curves conform to the requirements set for temperature alternations in chamber tests of Kentucky bluegrass, celery, and tomato seed (see p. 322)—that is, the temperature falls below 21.5° in less than 15 hours after it first rises above 20° and is below 22.5° for more than 12 hours and as low as 20 for several hours of the day. Besides, the approximate mean temperatures for the day (23° May 18 and 23.5° June 27) are just about the same as for some of the more successful alternations, as shown earlier in this paper, and are less than for the alternations Nos. 9, 10, and 11, which gave lower total germination, and in which the temperature remained relatively high during a large part of the day.

COMPARISON OF TEMPERATURE CONDITIONS GIVING MOST RAPID GERMINATION IN THE CHAMBERS WITH THOSE GIVING EARLIEST SEEDLINGS IN THE FIELD

The smallest number of days required for the production of seedlings in the field was 4 days for bean and muskmelon seeds, 5 days for beet, pea, squash, and tomato seeds, 6 days for watermelon seeds, 8 days for onion seeds, and 12 days for parsley seeds. The average daily range in the temperature of the soil at a depth of 1 inch during this period of most rapid seedling production was determined by adding separately the maximum temperatures and the minimum temperatures for the separate days and dividing the sums by the number of days. Table IV compares these temperature ranges with those occurring in the chambers in case of the temperature alternations giving most rapid germination and shows also in each case the approximate number of hours of each day during which the temperature remained below 22.5° C.

The average daily range of temperature for most rapid seedling production in the soil was in every case greater than for most rapid

germination in the chamber tests. With all except the cucurbits (muskmelon, squash, and watermelon) the average daily minimum was from 2° to 6° C. cooler in the soil than in the chambers. The average daily maximum was approximately the same in the soil as in the chamber in case of bean, beet, and pea seeds; 3° cooler in case of onion seeds; and 3.5° warmer in case of parsley and tomato seeds.

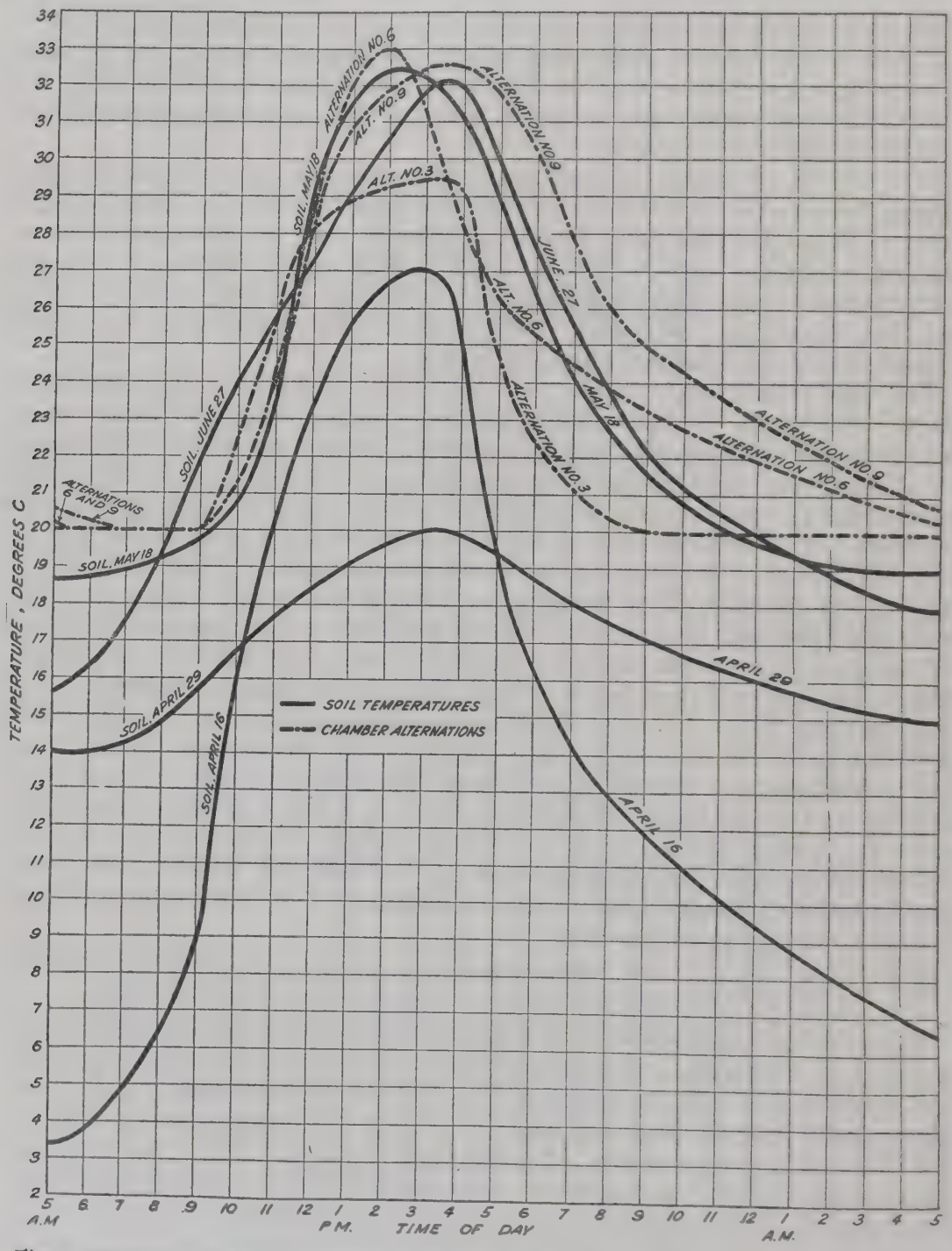

Fig. 20.—Curves showing soil temperatures for four days and chamber temperature with three alternations

With the seeds of the cucurbits the average daily minimum temperature of the soil when seedlings appeared most promptly was the same as or warmer than that occurring in the chamber tests, and the average daily maximum was 7° or 8° C. warmer than the maximum in the chamber test giving most rapid germination, although the mean temperature of this alternation was warmer than that of any other.

TABLE IV.—*Comparison of temperature conditions giving most rapid germination in the chambers with those giving earliest seedling production in the field*

Kind of seed.	Place of test.	Daily range of temperatures giving most rapid germination or earliest seedling production.	Number of hours below 22½° C.	Number of temperature alteration.[a]
		° C.		
Bean	Chamber	20 to 33	10	9
	Soil	18 to 32	12	.....
Beet	Chamber	20 to 33	10	9
	Soil	18 to 32	11	.....
Muskmelon	Chamber	20 to 33	10	9
	Soil	20 to 40	9	.....
Onion	Chamber	20 to 33	10	9
	Soil	14 to 30	16	.....
Parsley	Chamber	20 to 28. 5	14	5
	Soil	17 to 32	12	.....
Pea	Chamber	20 to 33	10	9
	Soil	16 to 33	14	.....
Squash	Chamber	20 to 33	10	9
	Soil	23 to 41	3	.....
Tomato	Chamber	20 to 28. 5	11	7
	Soil	18 to 32	11	.....
Watermelon	Chamber	20 to 33	10	9
	Soil	20 to 40	8	.....

[a] See figure 17.

CONCLUSIONS SUGGESTED BY THE FOREGOING COMPARISONS

Altogether Table IV and Figure 20 show a close correspondence between chamber temperatures and soil temperatures which gave most prompt germination of the seeds used. Undoubtedly a similar correspondence exists between the temperature conditions best fitted for artificial germination tests of Bermuda grass and Johnson grass seed (fig. 5 and 6 and text p. 304–305) and the soil temperatures during the period in which they germinate in nature in the warm climate of the places to which they are native.

The results of the comparison suggest:

1. For general use in conducting germination tests an alternation of temperatures covering a somewhat wider temperature range than the customary alternation between 20° and 30° C., for instance an alternation between 20° and 32° C. However, if the alternation is secured by the method of transfer between two germinating chambers at fixed temperatures, probably better results would be obtained with many kinds of seed by the use of a temperature range less than that characteristic of conditions in the soil, where the temperature changes are less rapid and the period during which the temperature is near either extreme is shorter. In view of these considerations and the additional fact that the warmer alternations Nos. 9 and 10 though giving more rapid germination in many cases, were less favorable in other cases than the cooler alternations, an alternation similar to No. 3 seems to meet the theoretical requirements of approximating with sufficient accuracy what may be termed optimal field conditions.

2. For use in germination tests of onion seed an alternation of temperatures covering a cooler range than that of any of the alternations with which it was tested in this investigation.

3. For the germination of seeds of the cucurbits an alternation of temperatures covering a warmer range than any of those investigated.

Whether or not any advantage would be derived from any of these suggested changes in method remains for further experiment to show.

SUMMARY

(1) Seeds of carrot, parsley, timothy, awnless brome grass, perennial and Italian rye grasses, meadow fescue, and several kinds of flower seeds germinate practically as well at favorable constant temperatures as with an alternation of temperatures.

(2) Seeds of redtop and parsnip and sometimes seeds of petunia germinate somewhat better, and seeds of celery, orchard grass, Kentucky bluegrass, Bermuda grass, and Johnson grass germinate much better with favorable alternations of temperatures than at constant temperatures. The exact alternation giving best results depends upon the kind of seed and to some extent also upon its physiological condition.

(3) Different lots of the same kind of seed sometimes vary widely in temperature sensitiveness. It may therefore be that some kinds which are usually constant-temperature germinators may, under certain conditions, germinate better with an alternation of temperatures. Incomplete after-ripening might have this effect.

(4) Several hypotheses have been suggested which may help to explain the effect of alternating temperatures upon germination, but none of them can be considered adequate without more definite evidence than is now available.

(5) The favorable effect of an alternation of temperatures upon the germination of certain kinds of seed can not, with our present knowledge, be referred to the specific effect of the extreme temperatures of the alternation or of the mean temperature of the alternation but are the result of the changes in temperature. In the case of Kentucky bluegrass and Johnson grass seed, at least in some cases, the effect of the mean temperature of the alternation supplements the effect of the alternation as such.

(6) On account of greater ease, simplicity, and uniformity of temperature control, coupled with equally good germination results, the method of securing temperature alternations by transfer between two germinating chambers at fixed temperatures is preferable to the method of heating and cooling a single chamber.

(7) In the use of temperature alternations the upper temperature should be maintained only a small part of the day, never more than eight hours and usually not more than six hours, and the change to the lower temperature should then be fairly rapid. These results are easily attainable by the transfer method.

(8) An alternation between 20° C. for 16 to 18 hours and 30° for 6 to 8 hours each day gives good results in the germination of parsnip, celery, redtop, and orchard-grass seed and seems to be an optimum temperature condition for germination of Kentucky bluegrass seed.

(9) A large percentage of at least some lots of celery seed will germinate at nearly constant low temperatures (15° to 20° C.), but germination is then so slow as to make the use of a warmer alternation preferable.

(10) A similar alternation between 20° and 35° C. gives good results with Bermuda grass seed; an alternation in a still warmer temperature range might possibly be still better.

(11) For the germination of Johnson grass seed the very warm alternation 30° C. for from 18 to 22 hours and 45° for from 2 to 6 hours each day is best.

(12) Those lots of Johnson grass seed which germinate most readily under favorable temperature conditions are less sensitive to temperature—that is, they germinate well under a wider range of temperature con-

ditions—than those lots which germinate less readily even under the most favorable conditions.

(13) A large percentage of some lots of Johnson grass seed will go through at least the first visible stages of germination and some of the seeds will develop apparently healthy seedlings at warm constant temperatures (35° to 40° C.), but a part of the seedlings are abnormal and capable of only slight development at these temperatures while the percentages of germination remain below those for favorable alternations of temperature.

(14) With alternations between 25° and 40° C. or between 30° and 45° the length of time from two hours to eight hours during which the seeds are kept in the warmer chamber has little effect on the percentage of germination of fully after-ripened Johnson grass seed but affects the rate of germination somewhat more.

(15) Removal of the scales from Johnson grass caryopses greatly increases their germination under relatively unfavorable temperature conditions, somewhat increases their germination with the most favorable temperature alternations, and considerably accelerates their germination in both cases.

(16) The temperature changes to which the seeds are subjected in any given alternation vary with their position within the germinating chamber and with the method of testing (whether between or on top of wet blotters) and are in no case exactly the same as indicated by a thermometer inserted into the air in the top of the chamber.

(17) The temperature changes giving best germination results with a large number of kinds of seed correspond rather closely with soil temperatures in the field under conditions which induce the most prompt and vigorous production of seedlings. A similar correspondence undoubtedly exists between the temperature conditions, giving best results with Bermuda grass and Johnson grass seed on the one hand and soil temperatures in the warmer localities to which these grasses are native on the other hand.

(18) The results of field tests suggest the use of an alternation between about 18° and about 32° C. for the germination of a large number of kinds of seed; an alternation covering a lower temperature range than 20° to 30° with onion seed; and an alternation covering a considerably higher temperature range with seeds of the cucurbits. Without experimental evidence, however, it is impossible to say whether or not any of these alternations in the cases indicated would have any advantage over an alternation between 20° and 30°.

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WATER CONTENT OF BARLEY KERNELS DURING GROWTH AND MATURATION[1]

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INTRODUCTION

In studies in kernel development during the last few years close records have been kept of a number of variables. Several of these studies already have been published.[2] Of all of the variables observed the behavior of water has been the most consistent. As shown in 1920,[2] the percentage of water by days from the date of flowering was practically identical in two different seasons at Aberdeen, Idaho, and showed almost no fluctuation. When plotted, the percentage was essentially a straight line starting at about 82 per cent and ending at about 48 per cent. Below 48 per cent the curves were not coincident. The uniformity of these results indicates a closely vital relation of water content with growth and maturation. The results previously reported have been based on the averages of a varying number of spikes per day. The wet weights of the kernels were secured individually, but the dry weights were obtained on the aggregate kernels of each spike. In the studies here reported the dry weight of each individual kernel was obtained, so that individual dry weights and percentages of water of individual kernels are available for the first time. For certain purposes these data have been totaled as before.

MATERIAL AVAILABLE

Several varieties were studied in detail. The earlier attempts were with hulled varieties. These were found to be unfit because it becomes impossible to remove the lemma and palet once mechanical drying has begun. In these varieties the sampling became impossible later than this period and, therefore, the final incidents of maturation were lacking. The results with naked varieties only are reported in this paper. The growth tables of three growing periods were available for interpretation. These include two seasons, 1919 and 1920, with the Baku variety, and one season, 1920, with the Jet. The Baku is a white, naked 2-rowed

[1] Accepted for publication May 20, 1922. These studies were made on the Aberdeen Substation, Aberdeen, Idaho, in connection with cereal experiments conducted cooperatively by the Idaho Agricultural Experiment Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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variety originating near the Caspian Sea. The Jet is a 2-rowed naked variety from Abyssinia which develops a very black pigment in the pericarp at maturity. As the occurrence of this pigment is thought to be of significance in the later stages of maturation, the Jet variety is considered the basic one in the discussion which follows. The growth

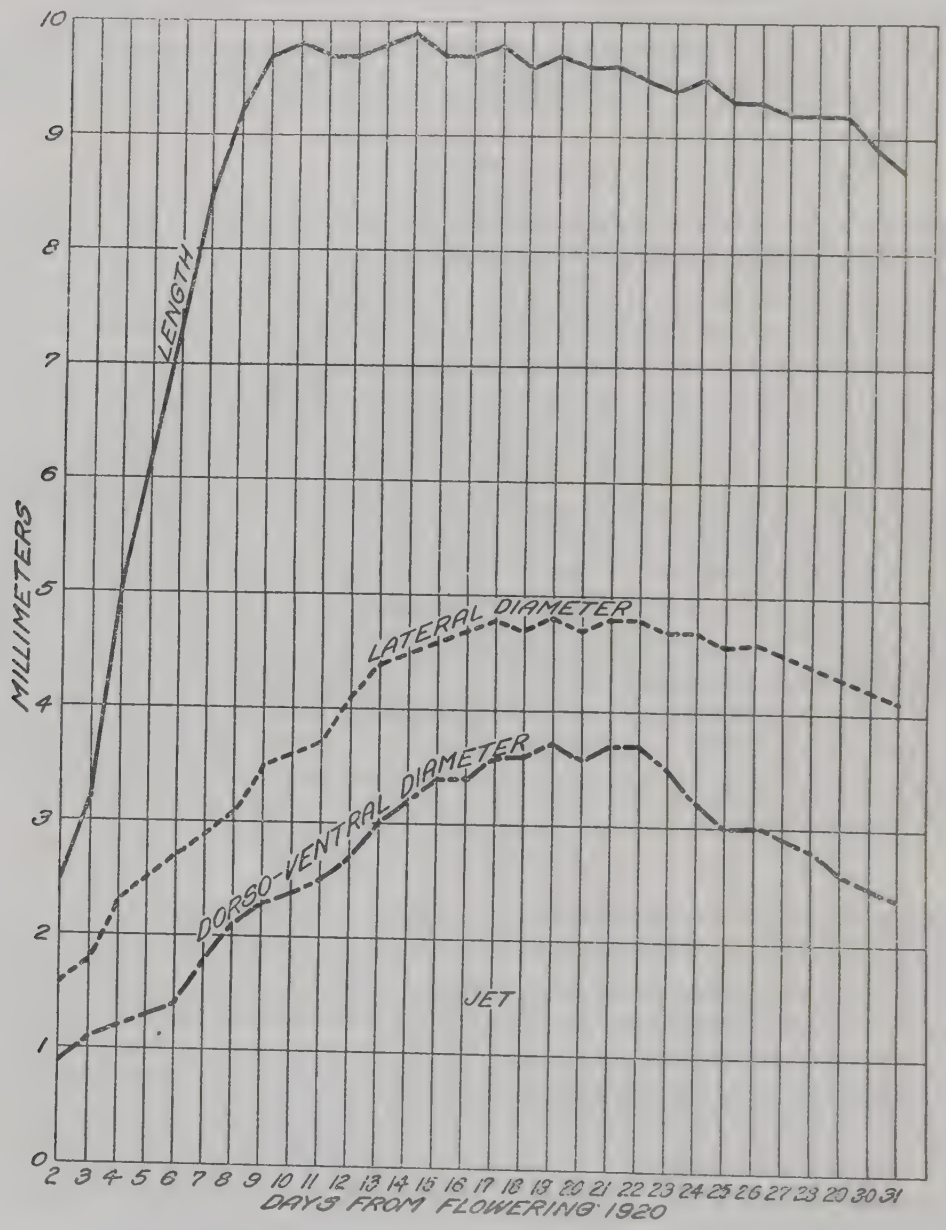

FIG. 1.—Average length, lateral diameter, and dorsoventral diameter of kernels of Jet barley at Aberdeen, Idaho, in 1920.

and maturation of the Baku variety vary somewhat from those of the Jet. These differences are apparent in the tables and figures and are pointed out in the discussion. In the figures which give data from the Baku variety for both 1919 and 1920, the time from flowering is for the 1920 data. The Baku variety in 1919 is advanced three days to avoid confusion of the lines. The shorter growing season of the Baku variety in 1919 was doubtless the direct result of drought, as the water for irrigation was insufficient in that year.

SUMMARY OF PREVIOUS INVESTIGATIONS

In papers previously published it has been pointed out that various phenomena occur with remarkable uniformity during the development of the barley kernel. The length, lateral diameter, and dorso-ventral diameter increase, attain their maximum, and decrease toward maturity

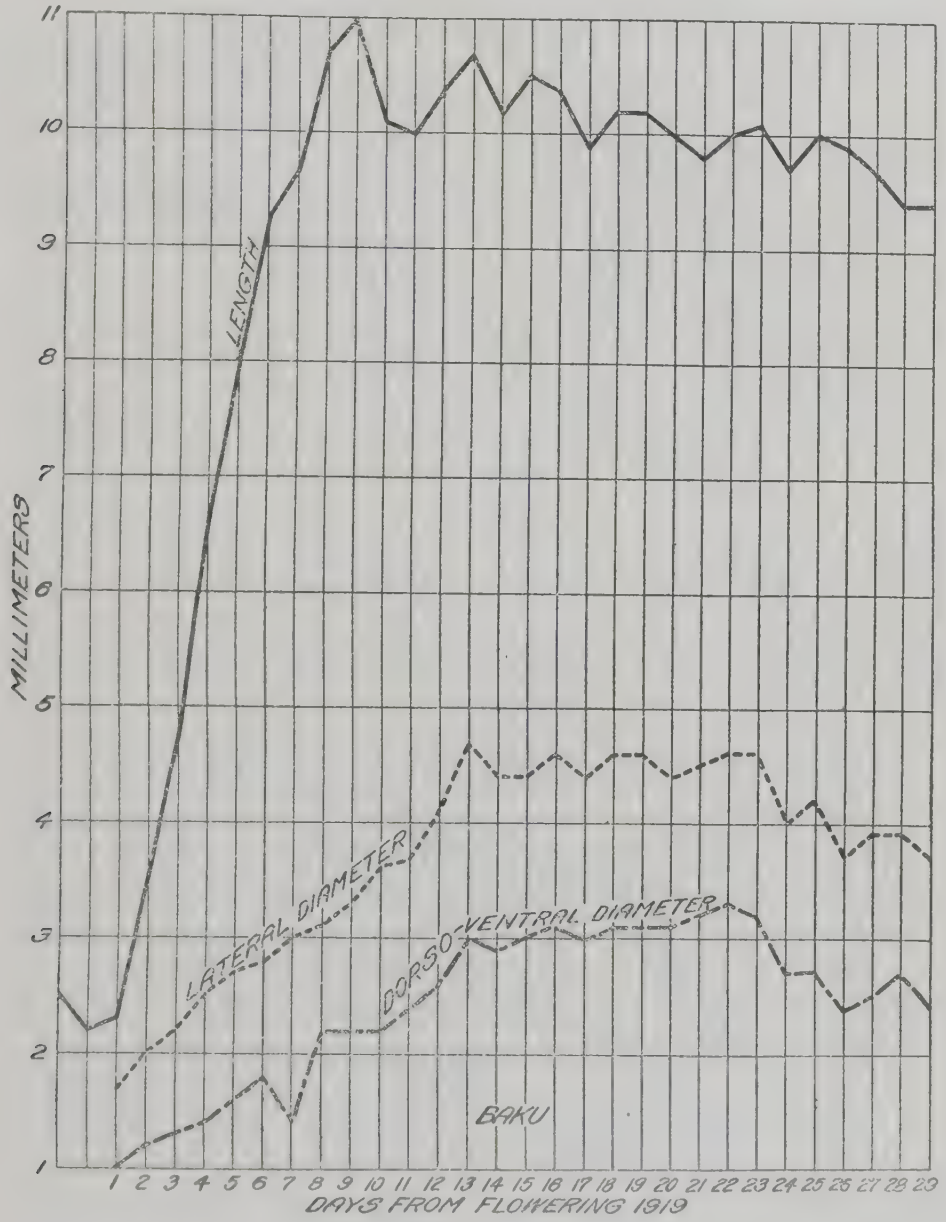

FIG. 2.—Average length, lateral diameter, and dorsoventral diameter of kernels of Baku barley at Aberdeen, Idaho, in 1919.

in very definite order. The wet weight increases rapidly and then decreases, as does the total water. The percentage of water decreases very uniformly from flowering to maturity. In the previous studies, where the unit has been the spike, maturation has occurred when about 42 per cent of water is present in the spike. This was necessarily an average figure which included kernels well past maturity and kernels which had not yet ceased to function. As may be seen in figure 1, the length of the Jet variety, in 1920, was reached by 9 days after flowering,

after which it remained almost constant until maturity. The lateral diameter had almost completed its growth by the fourteenth day and remained stabilized until the twenty-second day. The dorsoventral diameter increased until the eighteenth day, although the rapid increase was completed by the fifteenth day. The diameter was maintained until

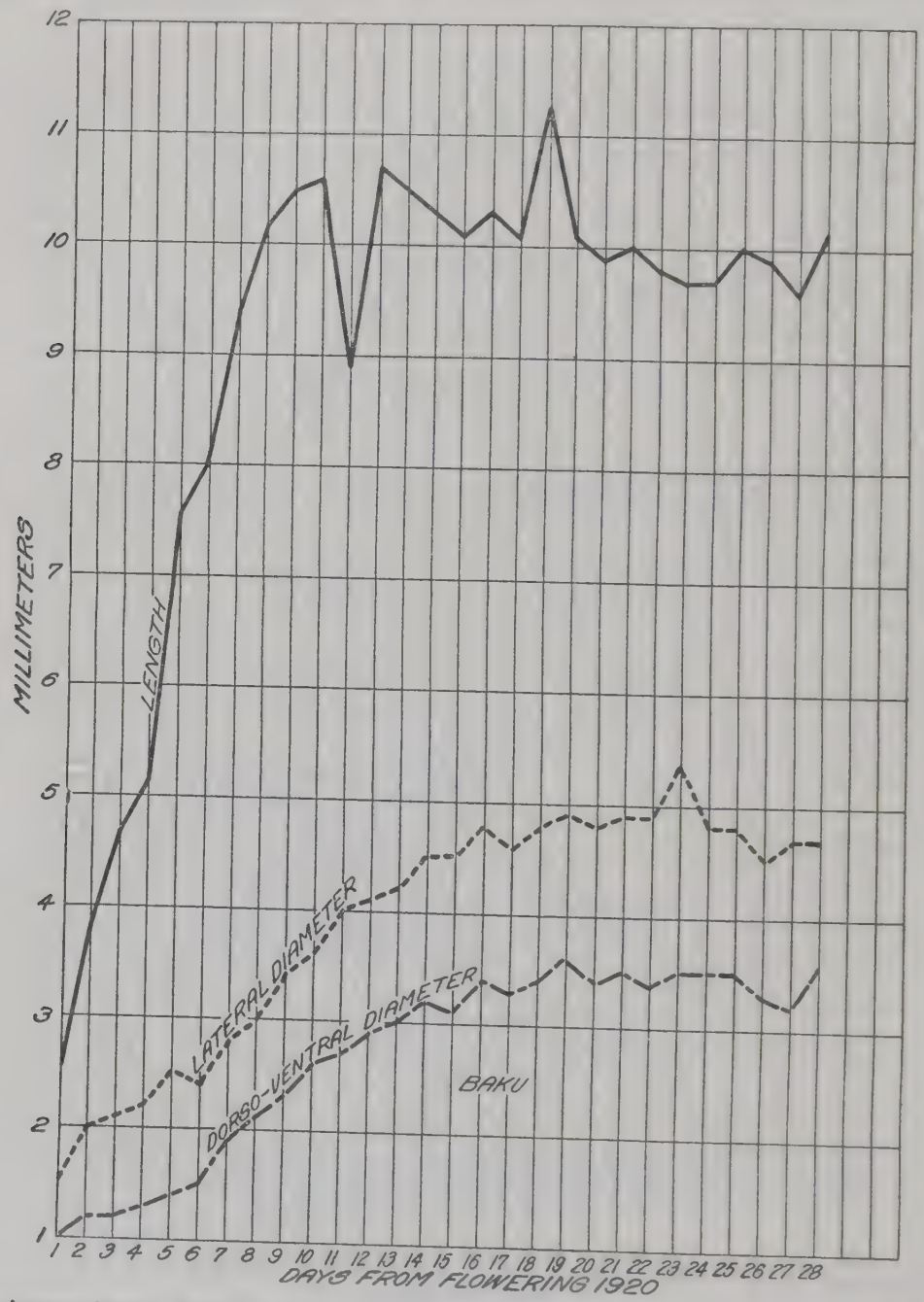

FIG. 3.—Average length, lateral diameter, and dorsoventral diameter of kernels of Baku barley at Aberdeen, Idaho, in 1920.

the twenty-second day. The wet weight increased rapidly for 17 days, with very slight continued increase until the twenty-second day. The total water increased rapidly for 16 days and showed no active loss until the twenty-second day. The dry weight increased rapidly until the twenty-second day and very gradually from then until the thirtieth. The percentage of water decreased gradually until the twenty-second day, rapidly from then until the twenty-seventh, and abruptly from the

twenty-seventh to the thirty-first, at which time the kernels had reached the moisture content normal to stored grain. As may be seen in Figures 2 and 3, the Baku variety reached these various stages at a slightly later date. In other words, the growing period of Baku was 2 or 3 days longer than that of Jet.

TOTAL WATER CONTENT OF JET AND BAKU VARIETIES

The average water content of the kernel of Jet, as in other varieties of barley, shows, when plotted, a very different curve from that of

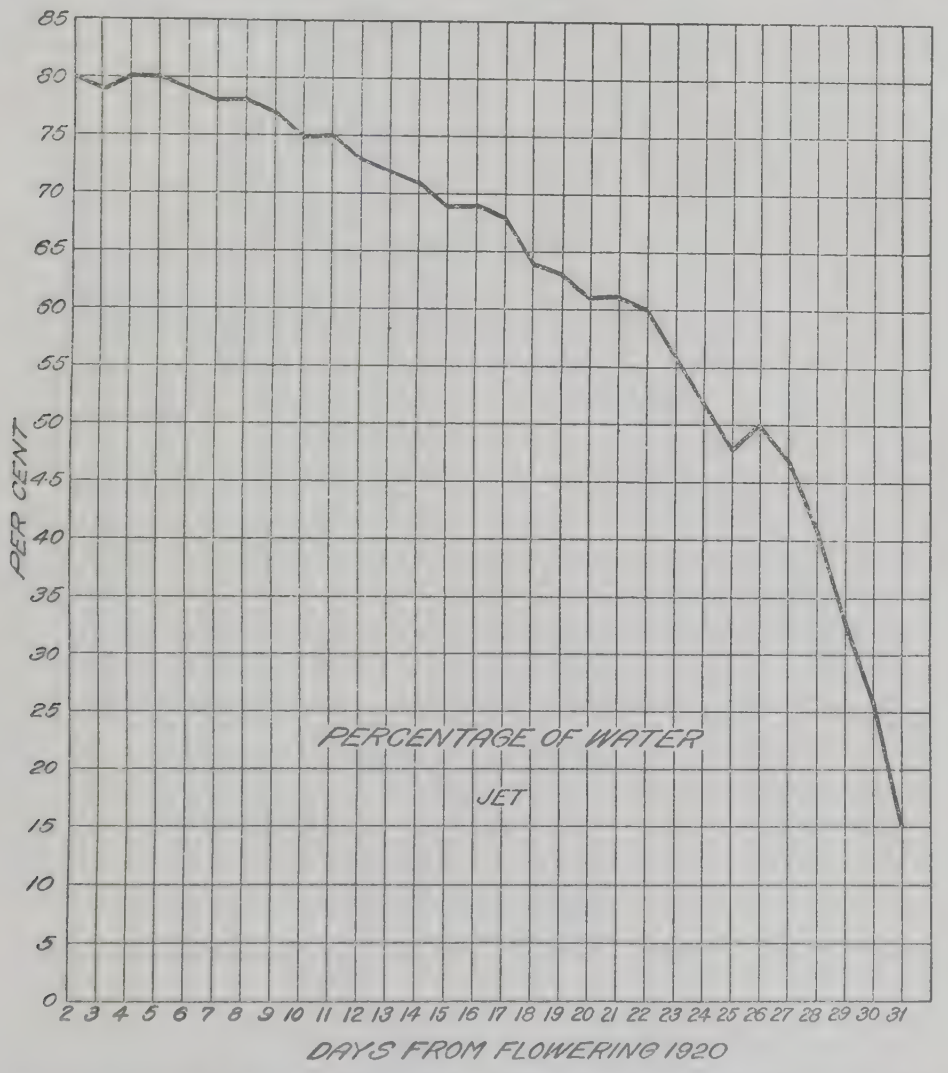

FIG. 4.—Average percentage of water in kernels of Jet barley from flowering to maturity at Aberdeen' Idaho, in 1920.

percentage of water. While not as perfect as in the Hannchen variety reported in 1920, the curve of percentage of water in Jet (fig. 4) descends in an almost straight line during the period of active growth. On the other hand, the curve of total water (fig. 5) exhibits three distinct phases. For a few days after fertilization there is a rapid increase in the water content of the kernel. There then follows a period of a few days in which the water content is almost stationary. Following this there is a uniform decline until maturity is reached and a more rapid decline to the point approximating the water content of the stored grain of the region. Stated in another way, for a period of about 12 days there is an increase

of water content simultaneous with a deposit of starch, the latter beginning the fourth day after flowering.

During the second stage there is little change in water content, but there is a heavy increase in starch content—that is, there is a deposit of starch which is not displacing water. The volume of the kernel

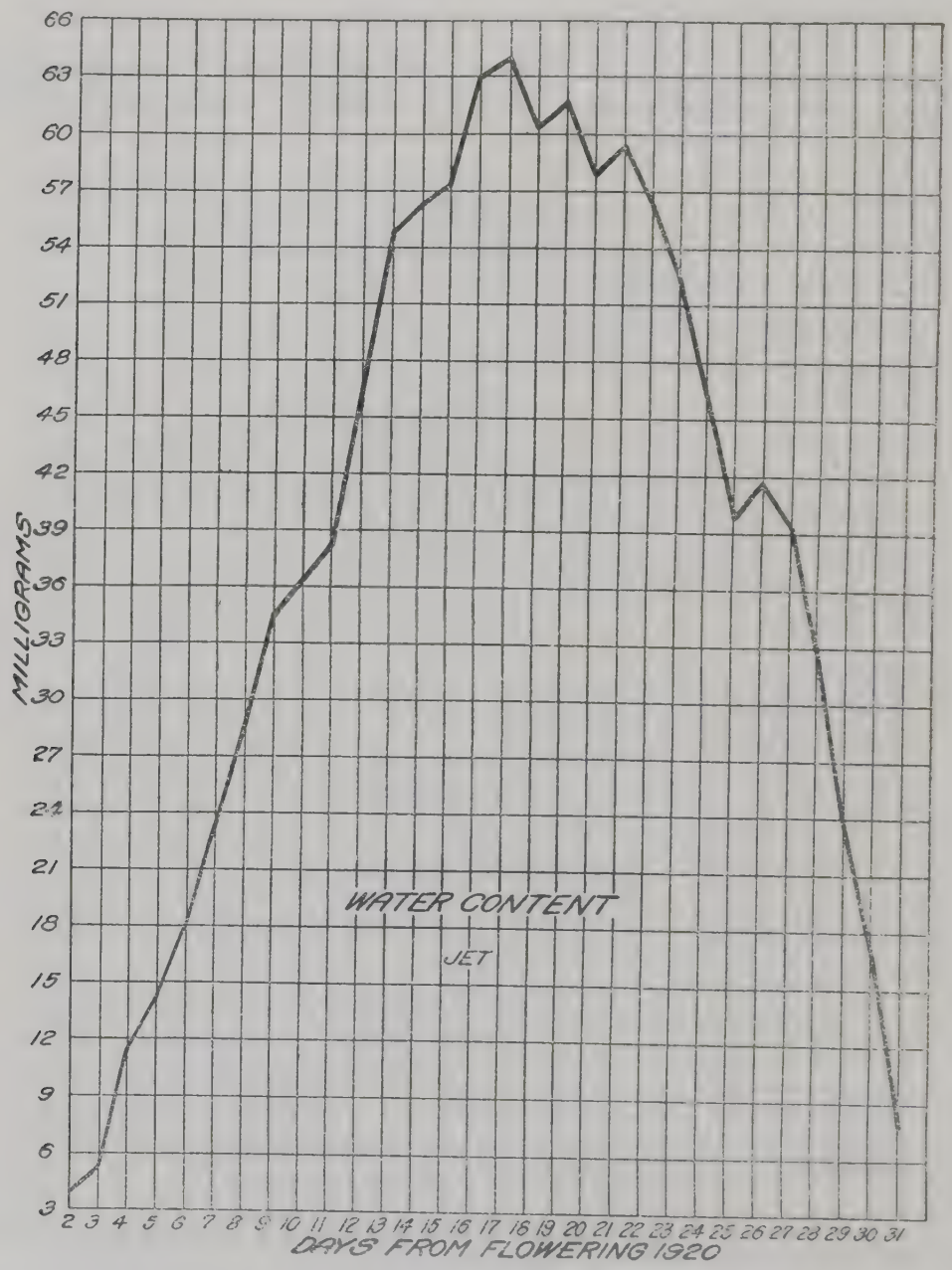

FIG. 5.—Average total water content of kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

continues to increase during this period. The increase in volume must come either from the increase in number of cells in the endosperm or from the increase in size of cells already formed. Most of the expansion is probably of the latter nature. The increase in volume is quite evident in figures 1, 2, and 3, which show the length, lateral diameter, and dorso-ventral diameter by days for both varieties.

The third stage, beginning about the twenty-second day in the Jet variety, shows a decided loss of water. While the rate of starch deposit

diminished as shown by the dry weight, material continued to be added for several days. The water content drops more abruptly and somewhat in advance of the wet weight, due, of course, to the continued activity of starch deposit.

The actual modifications which occur within the kernel during these changes have not been traced definitely. As the bulk of the kernel is endosperm tissue, the observable variations are doubtless dependent on a phase of endosperm development. The endosperm starts to develop about the center of the nucellus. As growth proceeds, new cells are added by repeated division of the endosperm cells first laid down. At some stage of growth the addition of these new cells is either diminished rapidly in the number added per day or, more probably, is interrupted

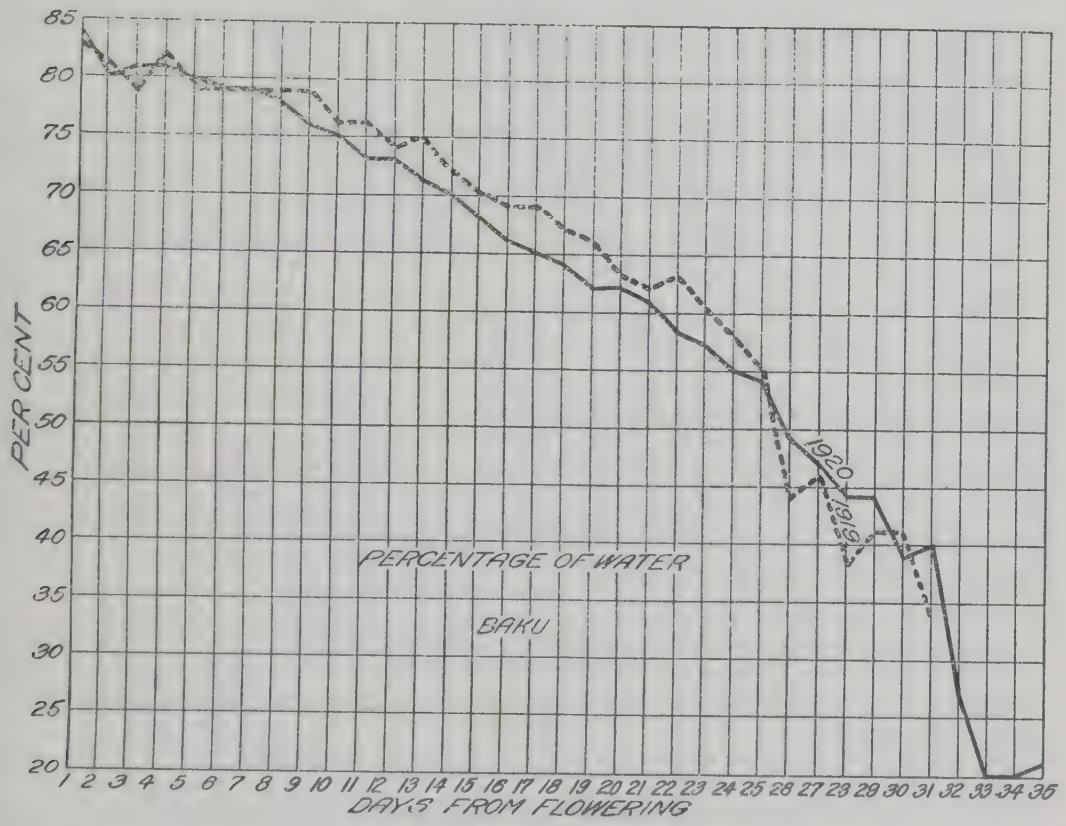

FIG. 6.—Average percentage of water in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920.

entirely except in the region immediately adjacent to the furrow. Later, two other developments are possible. As previously pointed out, there is a secondary stage of starch formation where small starch kernels appear among the large starch kernels of the first starch deposit. It is also likely that some of the cells of the starch endosperm are abandoned as places of active starch deposit. This certainly occurs in occasional cells of the periphery on the dorsal surface most remote from the furrow. Cells in the latter region can be observed which by their cell walls indicate an age equal to that of adjacent cells but which have very few starch grains in their interior. Such cells are, in a way, abnormal, and if there is any abandonment that occurs as a phase of maturation, it is of cells of normal starch content. In any case it is likely that the stages of endosperm development have a direct relation to the changes exhibited

in the wet weight, dry weight, and total water content of kerels. Judged by these indicators all varieties follow about the same course. Though Baku requires a day or two longer in maturing, its water content, as shown in figures 6 and 7, and its wet weight and dry weights, as shown in figures 8 to 11, are quite comparable to those of Jet.

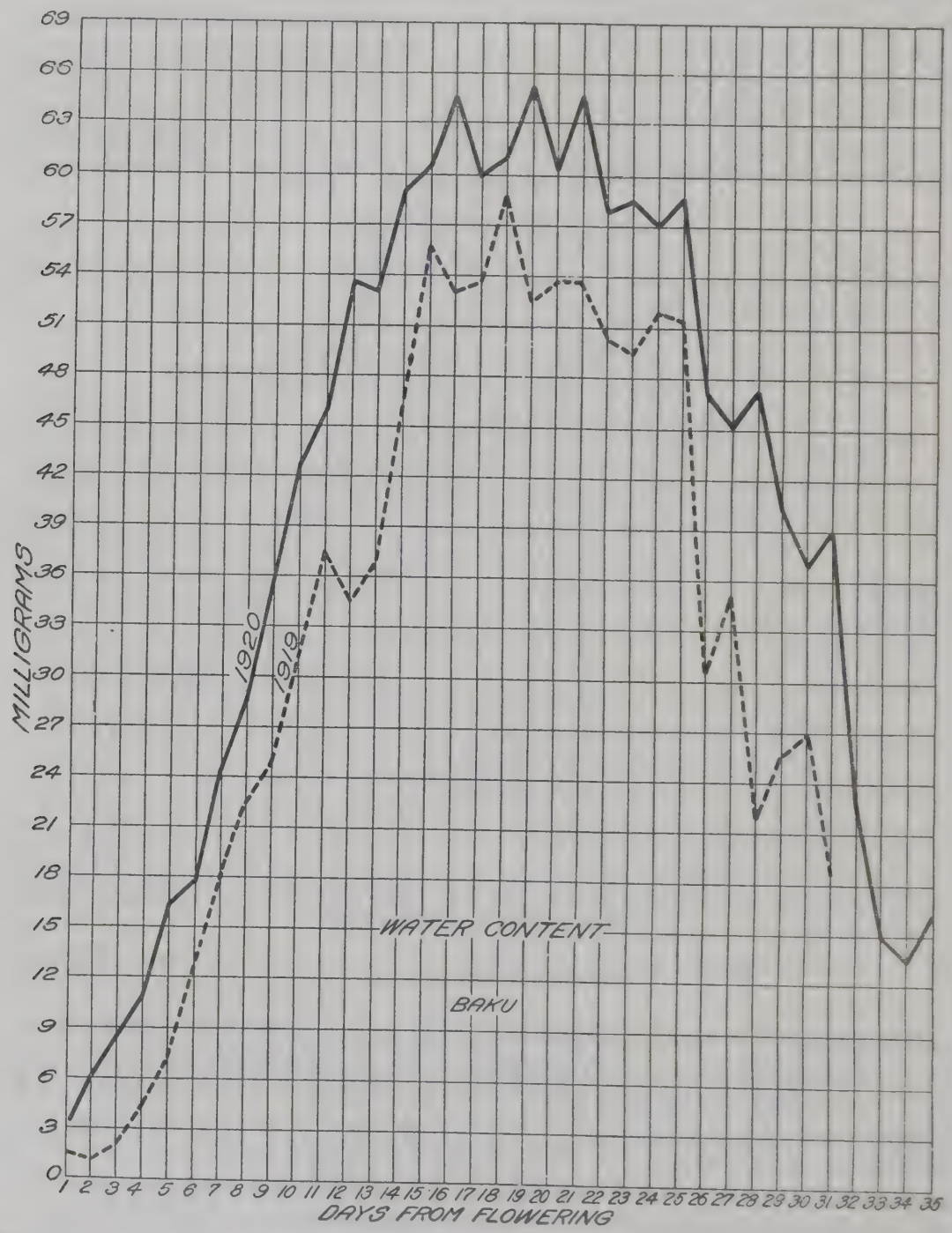

FIG. 7.—Average total water content in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920.

WATER CONTENT AND DRY WEIGHT OF INDIVIDUAL KERNELS

In Table I are given the complete records of the individual kernels of these two varieties for the period under observation. While the number of observations is so great as to make a direct digest of these data difficult, a number of facts are evident. In the table the kernels are

numbered on each spike. One side of the spike only was studied, as the error of sampling was less when a larger number of spikes were included, the daily number of observations being necessarily limited by the time required to obtain the data. The kernels are numbered from the base to the tip of the spike, No. 1 being the basal kernel, No. 2 the next above, and Nos. 6 to 11 the tip kernel, depending on the length of the spike. It

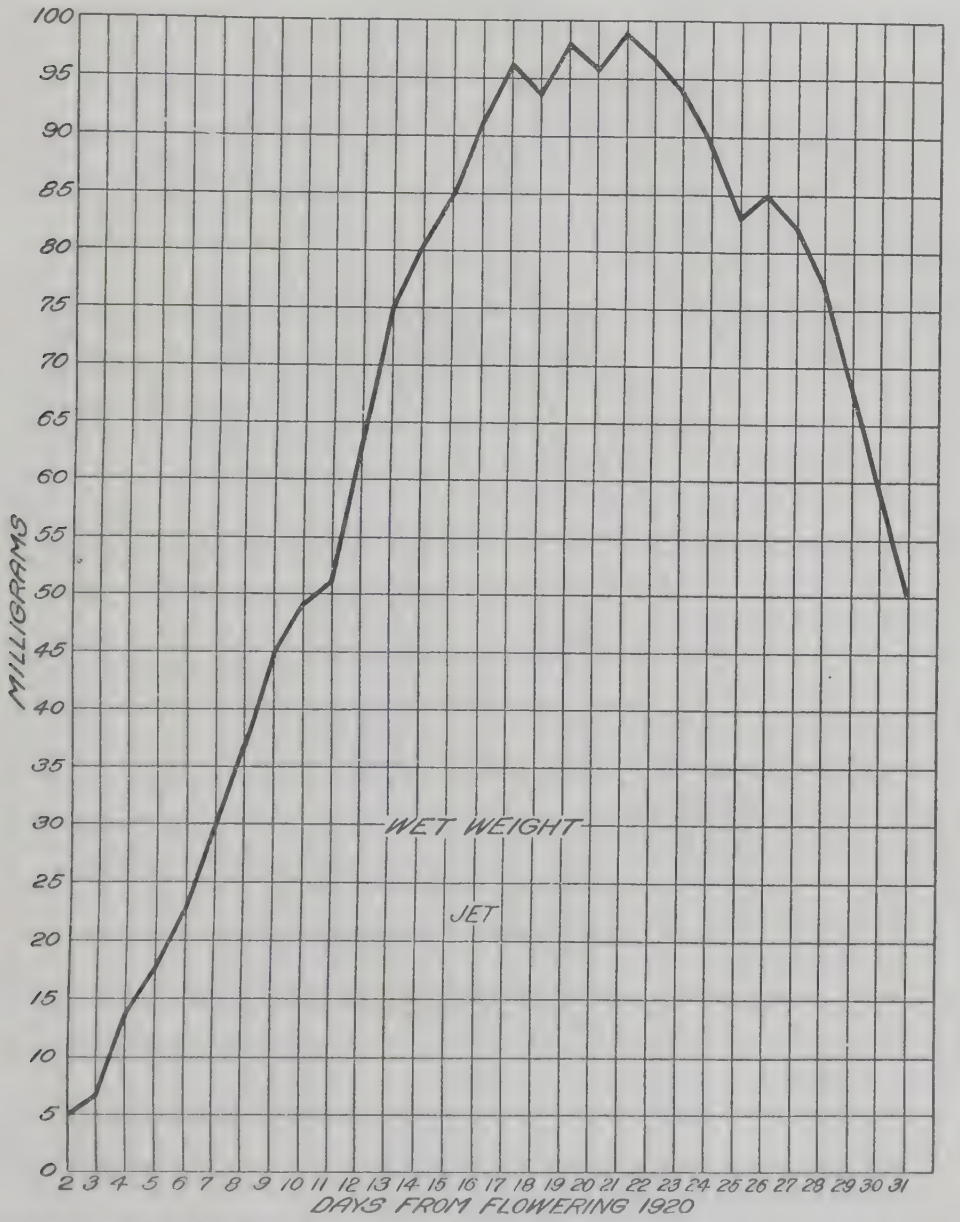

FIG. 8.—Average wet weight of kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

can be readily seen that the tip kernels never attained the size of those about the center of the spike. This fact has been frequently pointed out. As indicated by the percentage of water, it is quite noticeable that the kernels at the tip of the spike ripened much earlier than those near the base. The basal kernel itself is more nearly related to the tip kernels in that its average size is less than those above it and its maturation earlier. For the purpose of interpreting the table, any kernel under 42 per cent may be considered mature.

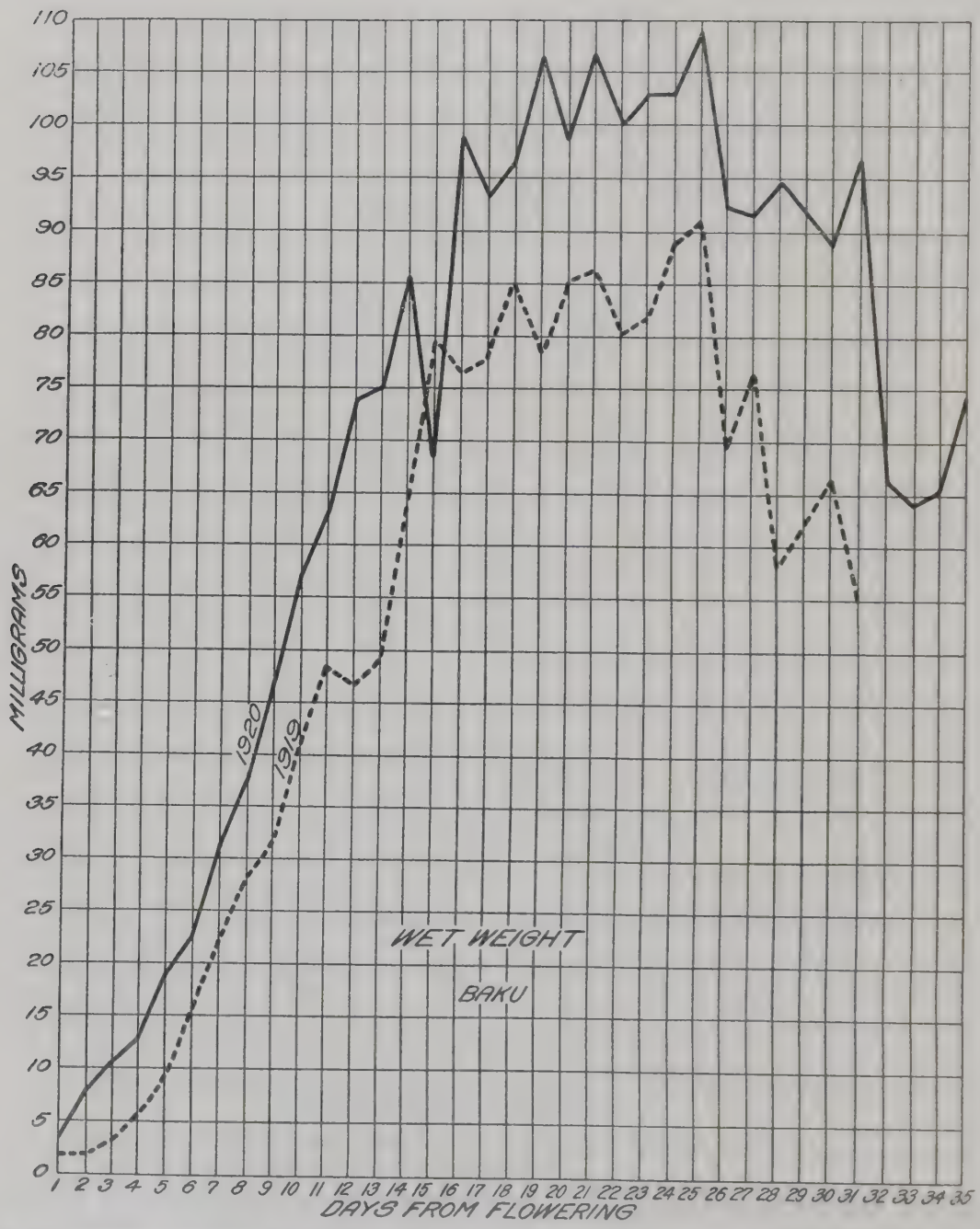

FIG. 9.—Average wet weight of kernels of Baku barley from flowering to maturity at Aberdeen, Idaho in 1919 and 1920.

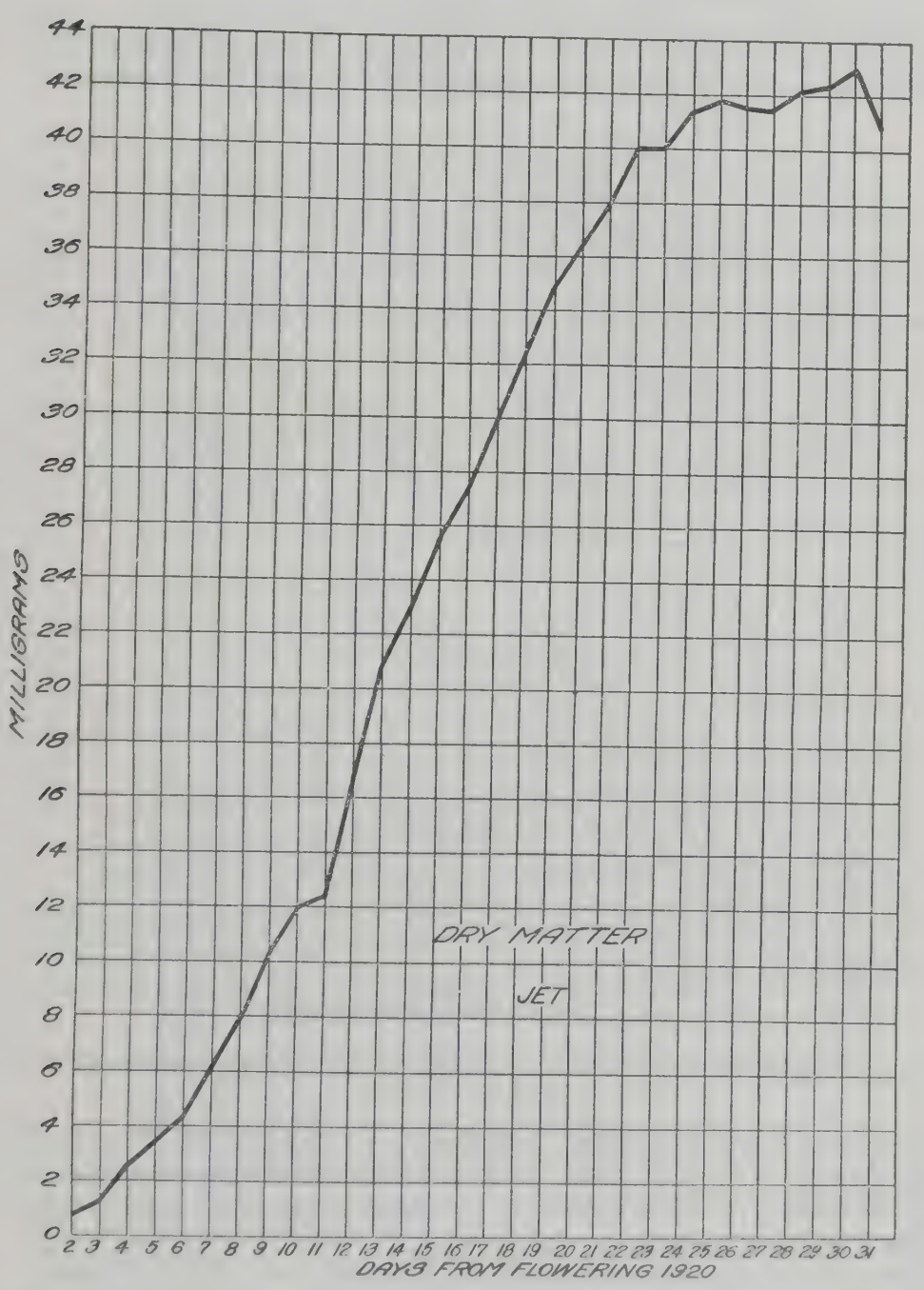

FIG. 10.—Average dry matter in kernels of Jet barley from flowering to maturity at Aberdeen, Idaho, in 1920.

Section 3 of the table differs from sections 1 and 2 in that the spikes of a single day are of different ages. It was thought that daily weather variations might be overcome if samples of varying ages were taken on each day. Spikes marked A before August 4 flowered on July 6, all those marked B, and those marked A after August 4, on July 7, and those marked C, D, and E, on July 9. In the figures showing the distribution by days from flowering and in the various averages, spikes

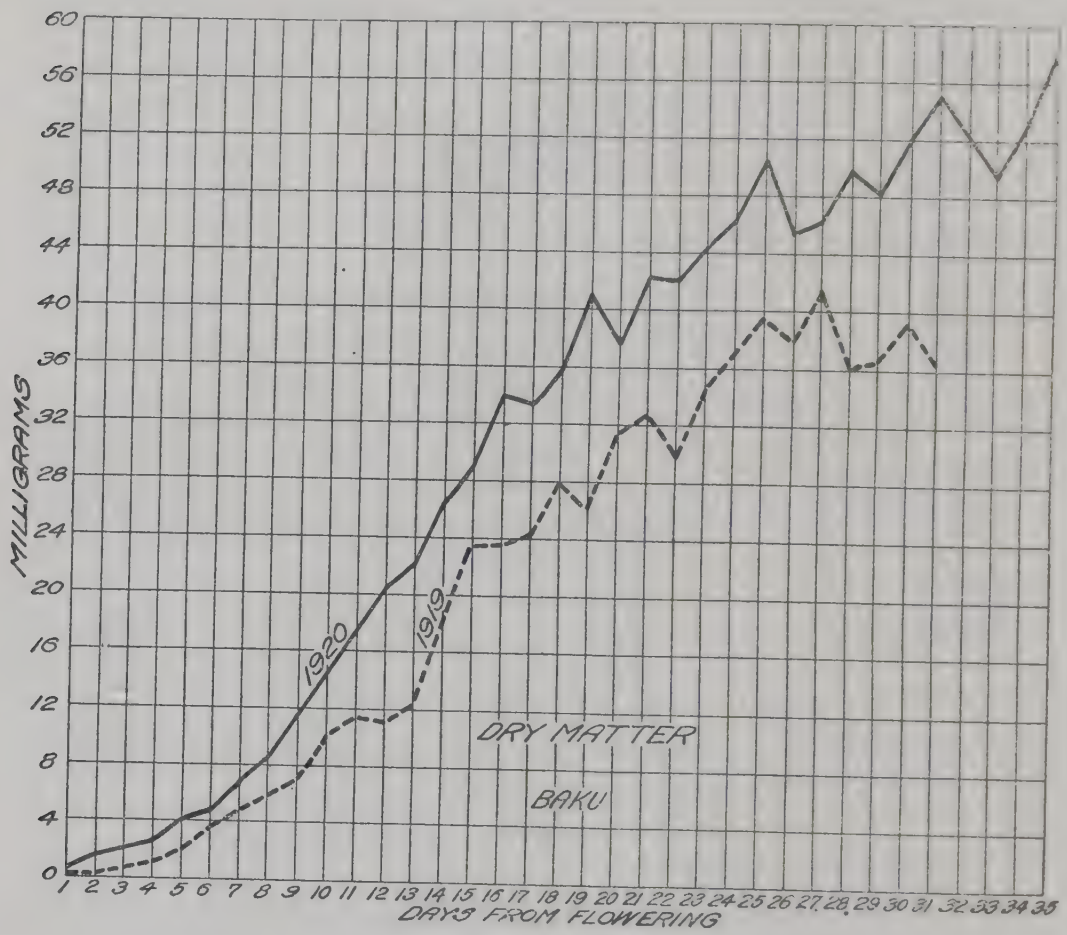

FIG. 11.—Average dry matter in kernels of Baku barley from flowering to maturity at Aberdeen, Idaho, in 1919 and 1920.

of the same age are grouped together. In computing data for figures, small abnormal tip and basal kernels were omitted where it was thought the abnormality was misleading. The data in the table have been plotted as frequencies in figures 12 to 15.

The frequencies of the dry weights (fig. 12) are not much more easily interpreted than the table itself. This is due to the fact that the final weights of the kernels on the spikes vary over a considerable range. The tip kernels begin to ripen about the twenty-first to twenty-third day and are found distributed to the left of the general mass of kernels from weights of 275 mgm. to 375 mgm.

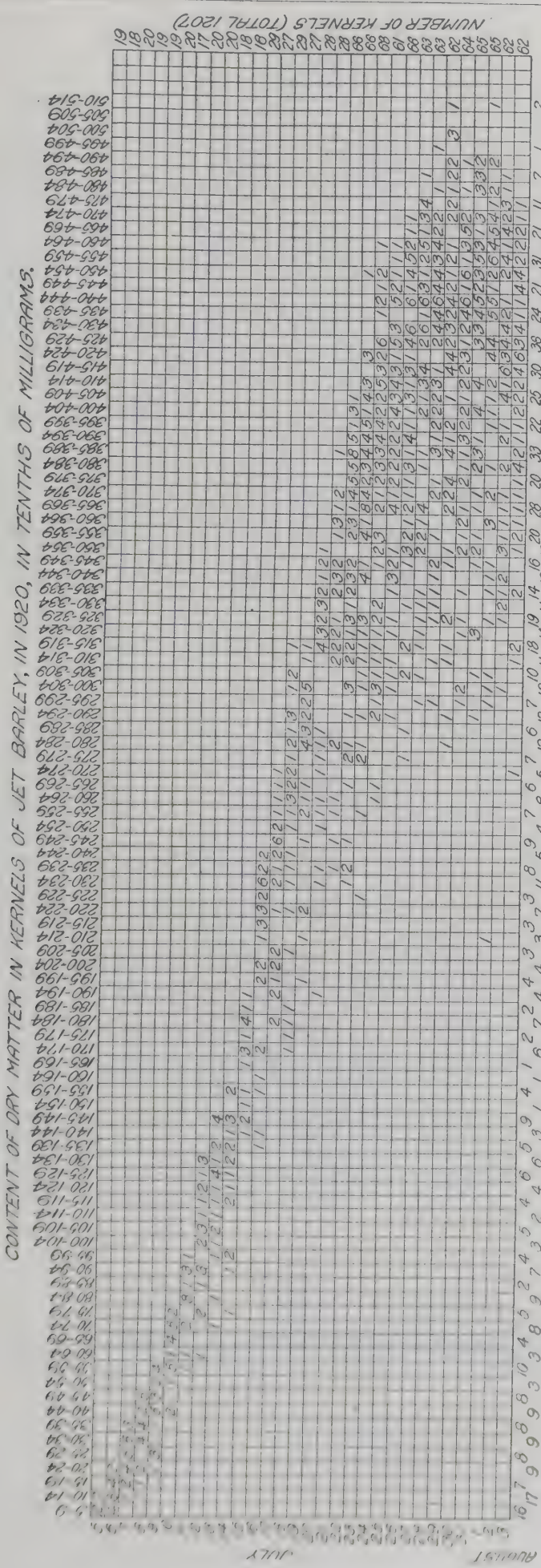
CONTENT OF DRY MATTER IN KERNELS OF JET BARLEY, IN 1920, IN TENTHS OF MILLIGRAMS.
NUMBER OF KERNELS
NUMBER OF KERNELS (TOTAL 1207)
JULY
AUGUST

FIG. 12.—Distribution of kernels of Jet barley according to the dry-matter content by day from flowering to maturity at Aberdeen, Idaho, in 1920.

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*

SECTION 1: JET, 1920

Date.	Spike.	Dry weight of kernels (mgm.).											Water in kernels (per cent).										
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
July 5	A	0.9	1.0	1.0	1.0	1.0	0.9	0.8	0.7	0.7	0.4	.....	81.2	83.3	82.5	80.0	79.6	81.2	78.9	76.7	72.0	73.3	.....
	B	.8	.9	1.0	1.1	.9	1.0	.9	.7	.7	.6	.....	84.0	83.3	81.5	80.7	84.2	81.1	81.2	82.5	75.0	73.9	.....
6	A	1.1	1.3	1.4	1.4	1.3	1.2	.8	.8	.5	.....	.....	79.6	81.4	80.0	80.6	79.0	76.0	76.5	73.3	76.2	.....	.....
	B	1.2	1.9	2.0	2.0	1.8	1.6	1.5	1.0	.8	.3	.....	78.2	79.6	80.0	79.6	79.5	80.2	77.6	77.8	75.8	83.3	.....
7	A	3.4	3.5	3.6	3.6	3.4	3.1	2.8	2.5	2.1	1.5	.....	80.9	79.5	80.2	79.7	79.9	79.7	80.0	79.5	79.0	77.6	.....
	B	2.3	3.1	3.0	2.8	2.5	2.6	2.3	2.0	1.7	1.3	.....	80.3	80.4	80.6	80.6	81.1	80.3	80.3	80.0	79.3	78.3	.....
8	A	4.0	4.7	4.9	4.8	4.6	4.5	4.1	3.5	3.0	2.3	.....	80.0	79.1	79.0	79.1	79.2	78.6	79.1	78.9	79.2	79.3	.....
	B	3.2	3.5	3.6	3.6	3.4	3.0	2.4	1.9	1.4	.....	.....	78.9	80.0	79.5	79.3	79.0	79.3	80.2	80.8	79.4	.....	.....
9	A	2.9	5.8	5.8	5.6	5.8	5.2	5.1	4.4	2.7	.....	.....	79.6	78.7	78.8	78.5	78.4	78.6	78.3	78.4	78.7	.....	.....
	B	4.4	4.9	4.8	4.4	4.7	4.4	4.3	3.8	2.8	2.2	.....	78.9	79.1	79.2	79.8	79.0	79.2	78.6	78.9	79.9	79.0	.....
10	A	5.6	7.1	7.6	7.5	7.2	7.1	7.1	6.6	5.8	5.3	4.2	78.0	77.9	78.2	77.9	77.9	77.7	77.6	77.5	78.5	77.6	77.4
	B	6.6	6.3	6.8	7.0	6.9	5.7	5.7	5.8	4.4	.....	.....	78.3	78.1	78.1	78.1	78.2	77.2	77.6	77.9	77.8	.....	.....
11	A	7.2	8.2	8.4	8.2	8.1	8.3	8.1	7.0	5.5	.....	.....	77.8	78.0	77.9	77.6	77.9	77.4	77.3	77.4	77.3	.....	.....
	B	8.0	9.2	9.5	9.4	9.0	8.7	8.0	6.4	.....	.....	.....	77.1	76.8	76.8	76.1	76.2	76.5	76.7	75.9	.....	.....	.....
12	A	9.2	10.8	11.0	10.7	10.4	10.2	9.3	8.5	7.5	.....	.....	76.9	76.5	76.4	76.7	76.4	76.5	77.0	77.3	77.8	.....	.....
	B	12.3	13.1	12.6	13.2	13.3	12.2	11.7	10.8	9.2	7.9	6.3	75.0	75.3	74.8	74.9	74.3	75.5	75.2	75.7	76.2	76.0	76.6
13	A	10.8	13.9	14.6	14.5	14.7	14.5	13.5	12.9	11.3	9.8	8.2	75.3	73.8	73.7	73.0	72.4	72.7	72.8	73.4	73.8	73.5	74.1
	B	11.6	12.3	13.2	12.9	12.9	12.8	10.5	10.0	7.1	.....	.....	75.0	75.5	75.2	75.0	75.0	75.0	75.3	74.9	75.2	.....	.....
14	A	11.5	13.1	12.7	13.8	13.1	12.0	9.8	9.4	7.7	.....	.....	75.5	74.8	74.5	74.1	74.1	74.4	75.7	75.5	74.4	.....	.....
	B	14.1	14.7	15.7	14.9	15.6	14.7	13.7	11.8	9.5	.....	.....	74.3	74.0	74.1	73.9	73.8	74.2	73.5	74.3	74.3	.....	.....
15	A	14.2	17.7	18.1	18.5	19.1	17.4	17.3	15.8	14.8	11.9	.....	72.3	72.1	72.4	71.9	72.4	72.6	72.3	72.6	72.2	72.8	.....
	B	16.6	18.0	18.3	18.3	17.2	15.4	14.6	.....	.....	.....	.....	73.2	73.5	72.6	72.9	73.6	72.9	73.9	.....	.....	.....	.....
16	A	24.4	24.0	23.5	23.2	22.6	22.1	21.8	19.8	17.4	15.6	.....	70.0	69.9	70.1	70.9	70.9	71.1	70.5	71.5	70.5	72.1	.....
	B	21.5	23.0	23.1	23.8	22.2	21.3	20.2	16.1	14.2	.....	.....	72.0	71.0	71.0	70.8	71.2	71.7	72.4	71.5	72.3	.....	.....
	C	20.4	22.3	23.2	23.1	22.8	23.1	21.7	19.8	17.1	13.6	.....	71.6	72.6	70.7	71.7	71.3	71.5	71.6	72.2	73.4	73.9	.....
17	A	25.3	25.8	26.5	27.0	26.3	24.8	23.6	20.5	.....	.....	.....	69.2	69.0	69.0	70.0	69.9	69.1	69.9	69.8	.....	.....	.....
	B	19.4	24.5	24.6	25.4	24.9	24.7	20.8	20.0	18.3	.....	.....	71.3	70.4	69.9	69.8	68.9	69.6	69.5	70.2	70.2	.....	.....
	C	19.0	23.3	24.3	24.4	24.6	23.2	22.3	19.6	18.2	.....	.....	71.7	71.7	71.5	71.0	71.3	71.4	71.4	71.6	72.3	.....	.....
18	A	17.8	26.4	28.2	26.0	26.6	26.4	25.1	23.1	17.2	.....	.....	69.4	68.2	67.1	66.9	67.8	67.8	67.8	67.5	67.1	.....	.....
	B	27.1	31.8	29.4	30.5	30.5	29.1	26.9	23.6	22.4	18.5	.....	68.0	68.1	66.6	68.6	67.2	68.2	67.2	66.8	67.0	66.5	.....
	C	28.4	29.0	30.2	28.9	27.5	27.4	25.5	24.1	20.9	18.0	.....	70.2	69.4	68.9	69.5	69.3	69.7	70.0	70.2	70.8	70.8	.....
19	A	28.7	31.3	30.0	31.8	30.2	28.8	28.0	26.0	22.0	.....	.....	68.9	69.1	67.7	67.9	67.9	67.9	69.0	68.0	67.4	.....	.....
	B	19.6	28.3	30.3	29.4	29.5	29.2	28.7	25.8	22.3	.....	.....	69.0	68.4	68.3	67.8	68.7	68.0	68.5	68.8	68.8	.....	.....
	C	24.5	26.5	28.4	30.1	30.2	29.6	28.1	25.5	21.1	.....	.....	69.7	70.7	69.3	68.5	67.9	68.2	68.0	67.6	67.1	.....	.....
[illegible]	A	.....	32.0	33.1	33.7	33.2	32.6	32.1	27.7	26.1	23.9	.....	68.6	67.4	67.8	67.6	67.2	68.1	67.7	67.9	68.0	.....	.....
	B	19.1	33.2	34.6	35.3	34.9	34.0	32.5	31.6	28.0	25.0	.....	66.4	67.1	66.8	66.6	66.4	66.4	66.1	66.7	66.6	65.6	.....
	C	28.6	31.7	33.5	32.3	31.6	31.8	27.2	25.8	23.3	.....	.....	67.2	66.6	67.3	67.3	67.0	67.1	66.7	66.8	66.5	.....	.....
[illegible]	A	28.0	33.6	34.3	33.9	32.6	31.4	28.4	24.9	.....	.....	.....	67.3	65.7	65.6	65.1	65.0	64.2	65.1	64.2	.....	.....	.....
	B	34.5	37.2	36.4	36.3	35.6	34.3	32.0	31.1	25.8	.....	.....	63.4	63.2	62.6	62.6	62.7	62.1	62.5	62.3	61.4	.....	.....
	C	31.5	36.2	38.7	37.3	34.4	36.8	34.6	32.2	31.5	26.3	23.6	64.0	64.4	63.5	64.0	64.1	63.3	63.3	64.7	63.3	64.0	62.9

22	A	.....	37.8	38.6	38.8	38.6	37.9	35.7	34.3	34.2	32.5	27.7	62.5	62.6	61.9	62.4	61.9	62.1	60.9	60.8	61.0	.....
	B	31.4	38.5	39.4	38.2	38.8	37.8	36.2	33.7	27.5	24.8	.....	65.8	64.9	64.2	62.7	64.0	63.1	63.2	63.4	60.4	.....
	C	37.9	40.0	39.2	39.6	38.0	37.2	36.0	32.9	31.6	28.3	.....	65.2	63.8	62.8	63.3	63.7	63.0	63.0	62.4	60.5	.....
	D	32.4	37.1	37.1	34.8	34.3	32.6	29.4	23.4	.....	.....	.....	64.0	64.0	64.0	62.9	63.2	63.2	63.3	63.3	61.2	.....
	E	33.6	38.2	39.4	38.9	38.9	34.9	31.3	30.1	26.5	.....	.....	64.6	65.0	64.6	65.2	63.7	64.6	64.3	63.2	.....	.....
	F	23.6	38.0	38.1	39.1	39.3	37.9	36.3	33.2	30.1	23.6	.....	62.4	63.5	61.0	61.7	62.1	60.9	63.4	63.0	61.7	.....
	G	40.2	40.2	40.6	38.6	37.3	39.3	35.5	31.8	30.0	.....	.....	62.2	62.7	62.6	62.8	62.1	62.4	62.3	63.1	63.0	60.7	.....
23	A	.....	37.2	40.6	40.1	42.4	39.6	38.0	36.7	35.5	30.4	25.7	63.4	61.9	61.7	62.0	62.4	62.1	62.3	61.2	59.6	.....
	B	41.0	42.2	41.3	39.1	39.0	37.0	36.9	34.2	31.9	27.9	.....	57.9	59.5	55.9	57.4	59.1	58.8	58.5	57.2	55.6	.....
	C	22.9	38.6	39.3	39.0	37.8	37.0	36.9	34.4	30.9	.....	.....	58.5	60.8	59.8	59.5	59.2	59.2	60.6	59.0	58.8	.....
	D	34.8	36.6	37.3	40.6	36.8	36.9	37.6	35.7	32.6	28.2	.....	61.2	60.5	59.8	59.9	58.5	59.2	60.6	61.2	60.8	.....
	E	39.6	42.2	45.3	40.6	40.9	38.6	38.7	36.9	34.4	32.5	.....	61.8	62.1	59.9	61.1	59.5	61.0	61.5	60.9	59.6	.....
	F	32.9	37.3	38.3	38.2	36.2	35.7	32.2	29.2	.....	.....	.....	62.9	63.2	62.7	63.1	60.7	63.3	62.7	61.6	59.6	.....
	G	36.5	41.0	40.9	39.7	39.9	35.6	34.2	31.0	27.6	.....	.....	60.7	61.2	60.7	60.2	60.3	59.4	60.0	59.7	.....	.....
24	A	.....	38.6	45.0	41.9	42.9	44.3	41.5	37.6	37.0	32.5	30.1	.....	62.1	61.3	60.2	60.3	59.4	60.0	59.7	58.3	.....
	B	38.4	41.4	42.1	41.5	42.6	41.3	39.5	39.6	33.2	34.5	.....	60.7	60.4	60.7	58.8	59.0	58.8	60.1	59.8	59.0	.....
	C	31.5	39.2	41.1	41.2	39.9	44.6	37.8	36.7	35.0	30.1	26.5	60.8	59.4	60.4	59.0	59.0	58.5	58.3	59.0	58.5	.....
	D	40.7	42.9	42.8	43.7	42.2	40.2	39.2	30.9	33.4	29.1	.....	61.6	61.5	61.2	58.1	62.0	60.0	60.2	59.2	58.8	58.1
	E	35.7	39.5	39.0	41.0	38.2	38.9	35.7	32.6	29.3	.....	.....	62.5	62.3	60.7	59.8	61.7	60.0	64.5	60.5	60.1	.....
	F	31.2	38.7	39.4	40.0	38.3	36.5	32.2	29.5	.....	.....	.....	62.6	63.0	61.7	61.8	60.3	61.6	60.5	.....	.....	.....
	G	30.3	42.6	42.6	46.0	45.1	44.1	35.3	40.8	35.6	26.3	.....	58.0	59.5	59.3	57.8	57.8	57.4	58.6	58.2	57.8	.....
	A	44.8	43.2	44.3	41.2	38.6	36.6	34.6	29.1	.....	.....	.....	58.4	58.8	59.1	57.7	57.8	57.6	57.2	57.8	57.2	.....
	B	40.9	44.5	45.8	44.3	43.4	42.5	40.4	36.5	30.5	.....	.....	60.0	59.7	58.4	57.7	58.6	57.8	58.0	57.0	.....	.....
	C	40.6	46.4	45.1	44.2	42.8	40.9	40.0	40.3	37.4	34.2	.....	56.7	57.8	57.4	56.5	56.7	56.3	56.1	55.5	54.7	.....
	D	42.6	44.1	43.0	42.6	39.8	41.0	38.0	34.7	32.0	.....	.....	57.2	57.4	55.7	57.8	57.0	58.1	57.5	59.9	.....	.....
	E	37.5	38.0	39.1	39.0	39.9	34.0	32.8	31.1	.....	.....	.....	59.6	59.7	59.8	58.4	58.1	57.8	58.2	58.1	.....	.....
	F	38.6	41.7	41.4	40.0	39.9	37.6	33.6	30.3	.....	.....	.....	60.7	60.4	59.6	58.8	58.8	57.8	58.4	56.7	.....	.....
	G	35.0	41.9	44.4	42.5	42.4	41.9	36.6	36.8	34.3	.....	.....	61.1	59.6	60.3	59.2	57.6	59.3	58.6	57.8	.....	.....
25	A	.....	37.1	45.4	45.9	45.2	43.4	39.4	38.4	36.8	28.8	.....	57.7	56.6	55.2	54.7	54.0	55.3	53.4	54.4	52.5	.....
	B	42.9	45.7	46.0	46.4	44.3	43.0	41.7	35.1	30.8	.....	.....	50.6	54.7	54.1	56.1	54.6	54.4	55.8	53.7	.....	.....
	C	40.9	45.1	44.0	43.1	43.2	42.4	38.3	34.8	.....	.....	.....	57.9	58.3	57.5	57.9	56.7	56.5	56.5	55.0	.....	.....
	D	43.3	47.1	46.9	45.8	45.1	44.2	40.4	39.0	35.7	30.9	.....	56.5	56.7	56.8	55.8	56.4	55.6	54.6	54.3	54.5	53.6
	E	37.9	42.5	44.1	42.6	42.8	41.1	38.7	35.5	31.8	27.7	.....	56.3	55.4	54.9	56.3	54.3	54.5	54.2	53.1	52.1	.....
	F	43.1	44.8	45.6	44.3	44.3	39.2	39.2	40.6	36.3	35.3	31.8	59.0	57.5	57.5	57.2	57.0	48.0	54.5	56.2	51.5	54.4	54.2
	G	36.8	39.6	41.6	40.9	41.7	38.2	35.2	33.3	.....	.....	.....	61.0	58.7	58.8	57.9	56.6	56.6	56.4	56.2	.....	.....
26	A	.....	32.9	43.2	44.0	44.9	43.1	40.3	36.9	33.1	.....	.....	54.7	54.0	52.7	53.0	53.1	45.0	51.9	52.0	49.2	.....
	B	44.3	48.8	47.0	47.8	44.3	46.0	41.9	35.8	30.8	.....	.....	52.1	53.2	49.6	52.2	50.8	48.6	50.2	49.1	51.0	.....
	C	41.8	46.2	47.2	46.4	46.0	44.8	42.8	40.6	35.6	35.2	.....	47.8	51.1	50.8	50.5	49.9	50.5	49.8	49.2	42.7	.....
	D	44.3	45.5	46.1	44.2	41.9	38.7	36.9	31.5	.....	.....	.....	51.6	52.7	51.6	50.2	47.5	48.4	48.5	49.8	.....	.....
27	E	43.0	45.1	45.7	43.2	42.8	36.3	36.7	29.9	.....	.....	.....	56.7	55.7	55.5	54.5	54.4	54.6	52.5	54.8	.....	.....
	F	41.1	44.1	46.1	43.2	44.0	41.9	41.0	38.0	35.0	32.2	.....	51.6	51.7	51.3	51.3	50.5	50.3	49.9	50.7	52.4	50.8
	G	43.4	47.9	47.2	47.7	46.7	43.6	41.4	36.6	33.6	.....	.....	55.7	55.1	53.9	55.0	55.1	53.8	54.2	53.6	52.5	.....
	A	44.7	47.4	48.4	44.3	45.0	40.8	43.2	39.4	34.7	29.5	.....	46.8	50.1	49.4	49.1	46.3	45.2	47.3	45.7	46.4	.....
	B	46.2	49.5	46.5	44.1	44.4	43.0	42.6	38.5	37.4	32.9	.....	48.1	46.4	44.6	44.2	43.4	43.3	43.9	44.8	41.2	46.3
	C	45.0	45.7	46.5	45.0	44.2	41.3	39.7	34.7	.....	.....	.....	54.1	52.5	51.7	48.9	48.1	46.8	45.7	42.7	.....	.....
28	D	43.0	46.1	46.1	41.0	43.8	42.8	40.8	38.6	33.5	.....	.....	51.1	50.0	49.0	44.8	46.5	44.8	46.1	51.7	51.8	50.6
	E	44.8	44.5	45.8	43.7	43.8	40.4	37.5	34.0	.....	.....	.....	52.7	51.7	49.4	47.7	47.7	47.3	47.4	45.9	.....	.....
	F	33.3	45.2	47.2	46.1	44.2	40.3	39.5	37.2	31.2	.....	.....	41.8	51.3	47.8	49.3	46.0	46.5	48.5	47.5	.....	.....
	G	41.5	44.4	44.8	43.2	43.6	41.4	38.6	35.7	.....	.....	.....	51.3	51.8	51.1	49.0	46.9	49.3	49.2	46.7	.....	.....

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*—Continued

SECTION 1; JET 1920—continued.

Date.	Spike.	Dry weight of kernels (mgm.).											Water in kernels (per cent).									
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
	A	38.7	42.4	43.3	41.8	41.9	36.9	37.3	35.2	29.2	.....	.....	51.9	50.0	50.1	46.3	47.4	41.6	48.6	47.8	48.3	.....	.....
	B	48.8	51.4	50.2	49.4	47.2	44.0	38.9	37.6	.....	.....	.....	53.1	52.6	52.2	51.6	52.7	50.4	49.7	49.7	.....	.....	.....
July 29	C	49.2	50.4	50.0	48.7	45.8	42.8	37.7	39.9	.....	.....	.....	52.9	52.6	53.0	53.1	50.5	50.3	50.4	49.2	.....	.....	.....
	D	37.9	46.2	47.1	45.6	44.5	41.5	40.6	38.9	31.7	.....	.....	59.2	54.4	51.3	52.7	52.9	52.7	51.3	51.5	50.8	.....	.....
	E	44.4	45.4	44.2	43.0	42.1	42.7	39.9	37.6	32.8	.....	.....	53.1	52.4	49.1	45.5	41.0	48.4	46.8	51.9	51.1	.....	.....
	F	47.8	48.2	47.6	43.2	44.6	44.4	43.5	38.7	36.8	32.9	.....	50.1	49.7	48.8	40.7	45.5	45.8	48.9	48.3	49.3	50.1	.....
	G	31.2	41.8	42.0	42.2	43.6	39.0	37.2	34.9	28.3	.....	.....	50.0	50.4	48.1	47.3	42.6	42.6	39.1	39.4	40.5	.....	.....
	A	45.1	46.8	45.4	45.9	45.1	44.0	39.1	35.1	32.7	.....	.....	52.0	51.0	50.6	50.7	49.9	51.3	50.9	48.3	49.8	.....	.....
	B	44.0	47.2	46.1	45.2	40.9	40.2	38.1	36.9	29.8	30.4	.....	48.5	46.8	46.3	42.2	43.5	40.4	37.2	41.5	36.5	39.3	.....
	C	39.7	46.8	46.7	43.7	45.1	44.9	44.3	43.5	39.6	33.6	.....	46.3	46.1	46.1	41.7	40.8	42.9	46.9	45.2	48.3	44.0	.....
30	D	37.6	43.7	43.4	42.5	42.2	40.0	36.0	.....	.....	.....	.....	54.3	54.2	53.4	52.9	52.4	52.0	49.7	.....	.....	.....	.....
	E	32.0	48.0	47.3	46.7	43.1	44.1	43.6	41.8	39.0	36.0	30.2	55.3	49.9	48.4	47.8	44.9	44.9	46.8	44.8	45.6	46.5	41.0
	F	41.3	44.1	42.2	37.7	41.6	42.1	38.6	35.8	.....	.....	.....	53.8	53.4	52.9	50.8	49.4	53.6	49.8	53.5	.....	.....	.....
	G	49.4	46.7	46.0	46.0	45.2	44.1	41.3	39.0	35.1	.....	.....	19.3	49.9	48.1	47.6	48.5	46.9	46.6	47.1	46.0	.....	.....
	A	36.8	46.2	44.4	43.7	43.1	43.7	41.2	39.0	34.6	.....	.....	35.4	44.6	44.5	45.9	42.4	43.2	42.5	41.1	34.0	.....	.....
	B	30.6	48.8	49.4	48.1	48.8	45.2	44.0	43.7	41.1	35.1	.....	30.4	38.3	46.2	45.1	46.0	43.3	43.1	43.2	43.6	39.5	.....
	C	44.5	45.5	42.6	42.6	40.3	40.4	38.6	35.6	32.2	.....	.....	32.4	31.5	28.2	26.9	24.0	24.9	25.6	25.0	25.5	.....	.....
31	D	38.4	48.6	46.8	47.3	43.4	45.8	45.6	42.9	41.0	37.0	.....	45.4	47.4	46.5	47.7	43.9	46.7	46.0	47.2	47.4	43.7	.....
	E	45.4	47.4	47.0	44.6	45.4	44.4	40.0	38.4	32.0	.....	.....	46.8	48.0	44.9	44.8	45.2	46.4	45.0	45.1	36.0	.....	.....
	F	43.8	44.0	45.7	44.3	43.3	40.0	38.8	38.5	35.0	29.3	.....	39.4	39.1	39.3	40.7	42.0	39.6	36.0	37.7	38.6	26.4	.....
	G	32.1	46.3	49.4	48.3	48.4	46.0	45.9	41.3	.....	.....	.....	47.4	45.3	49.4	48.8	49.8	45.6	47.7	46.5	.....	.....	.....
	A	49.4	51.0	49.3	46.0	45.6	47.0	42.9	42.4	39.7	33.7	30.9	41.8	42.8	40.5	39.1	38.3	38.7	26.8	21.2	18.0	17.4	21.6
	B	37.4	45.6	45.9	47.3	46.9	46.8	45.5	42.2	38.3	36.1	.....	37.5	41.8	43.5	42.3	38.3	37.9	39.4	37.9	36.8	38.8	.....
	C	30.0	47.8	46.4	46.9	44.7	42.6	42.2	42.4	40.0	.....	.....	20.6	38.6	37.0	33.9	21.0	20.8	18.7	15.4	18.4	.....	.....
Aug. 1	D	21.2	42.8	45.6	45.2	43.8	43.6	44.0	42.8	34.3	36.0	.....	20.6	28.8	31.9	28.6	27.1	22.6	26.7	30.5	28.1	22.2	.....
	E	40.8	48.1	48.4	46.4	46.6	45.8	44.0	43.6	41.4	.....	.....	14.8	36.6	33.1	23.7	31.0	37.3	31.4	33.6	25.9	.....	.....
	F	44.4	47.2	46.5	46.1	45.1	43.7	37.4	36.3	32.5	.....	.....	29.4	37.1	37.5	38.6	35.5	34.8	37.5	36.1	38.2	.....	.....
	G	29.5	47.3	44.4	44.4	43.9	41.2	34.7	.....	.....	.....	.....	37.6	45.9	43.9	44.8	41.9	37.6	28.9	.....	.....	.....	.....
	A	42.7	35.5	42.9	42.6	41.9	40.7	36.9	32.5	.....	.....	.....	25.2	21.5	16.5	19.9	15.7	15.9	16.7	18.9	.....	.....	.....
	B	35.3	47.9	45.8	48.8	47.1	46.5	42.1	40.7	35.4	.....	.....	32.2	39.6	37.8	40.9	42.0	42.7	40.5	41.5	35.9	.....	.....
	C	43.3	46.1	45.7	45.0	43.2	41.7	40.5	38.3	34.2	.....	.....	16.9	26.8	20.4	20.8	16.6	13.5	13.1	12.2	11.9	.....	.....
2	D	42.6	43.6	42.4	43.6	41.1	39.3	38.0	35.0	.....	.....	.....	18.5	24.6	22.3	19.7	18.8	14.9	13.8	13.4	.....	.....	.....
	E	47.0	48.1	47.8	46.6	43.0	41.8	39.0	33.8	.....	.....	.....	39.2	39.6	40.4	32.4	31.5	28.8	23.4	18.7	.....	.....	.....
	F	33.0	47.6	45.9	46.9	46.6	41.6	41.6	40.8	37.0	33.2	.....	29.2	32.3	30.9	29.6	22.3	18.4	15.8	14.1	13.5	13.3	.....
	G	43.0	45.2	45.8	44.0	42.4	41.5	39.8	36.1	30.3	.....	.....	30.3	36.6	35.6	34.7	35.2	26.2	29.2	23.8	15.6	.....	.....
	A	43.8	44.5	44.0	42.2	41.9	39.3	38.2	35.8	27.4	.....	.....	12.7	12.6	11.6	11.3	10.8	10.3	10.1	10.3	11.0	.....	.....
	B	41.5	44.6	41.4	40.0	40.6	37.6	33.7	31.7	.....	.....	.....	22.6	25.3	18.2	15.8	15.2	15.7	12.9	12.4	.....	.....	.....
	C	41.7	47.5	46.5	42.0	42.5	42.5	38.6	36.1	.....	.....	.....	18.4	22.9	22.0	14.6	13.4	14.7	13.6	12.6	.....	.....	.....
3	D	35.9	44.8	44.5	43.0	45.3	43.4	43.2	41.4	36.6	33.6	.....	14.9	21.4	17.7	19.9	16.1	14.4	15.0	11.7	11.6	10.9	.....
	E	40.7	45.3	45.8	38.4	42.2	41.6	38.3	38.5	31.2	.....	.....	13.4	20.2	16.4	19.8	13.0	12.2	11.3	10.9	10.9	.....	.....
	F	37.2	46.5	46.3	45.9	42.3	42.6	42.0	40.4	46.4	31.8	.....	14.5	17.7	17.6	14.5	13.3	13.1	12.1	12.0	.....	11.4	.....
	G	38.3	45.2	47.3	45.2	43.2	42.0	39.6	35.2	.....	.....	.....	12.9	19.0	19.3	19.4	19.7	15.8	11.4	11.3	.....	.....	.....

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*—Continued

SECTION 2: BAKU, 1919

July	1	A	0.3	0.3	0.3	0.3	0.3	0.2	.....	.....	.....	.....	.....	75.0	80.0	80.0	81.3	78.6	80.0	.....	.....	.....	.....
		B	.1	.2	.2	.3	.3	.2	0.3	.....	.....	.....	.....	90.0	86.7	87.5	82.4	81.3	86.7	80.0	.....	.....	.....
	2	A	.2	.3	.4	.5	.5	.4	.3	0.3	.....	.....	.....	83.3	81.3	81.8	78.3	77.3	81.8	81.3	78.6	.....	.....
		B	.2	.3	.3	.4	.3	.3	.2	.2	0.3	.....	.....	83.3	80.0	76.9	81.0	85.0	81.3	85.7	80.0	70.0	.....
	3	A	.1	.2	.3	.5	.4	.8	.2	.....	.....	.....	.....	83.3	80.0	83.3	79.2	75.0	77.1	75.0	.....	.....	.....
		B	.2	.5	.8	1.0	1.0	.6	.5	.3	.....	.....	.....	66.7	75.0	76.5	78.3	78.3	78.6	78.3	75.0	.....	.....
	4	A	.1	.2	1.1	1.7	2.0	1.8	1.4	.....	.....	.....	.....	83.3	90.0	82.3	81.1	80.0	79.5	79.4	.....	.....	.....
		B	.5	.7	.7	1.4	.2	.8	.6	.2	.....	.....	.....	83.3	84.1	84.1	79.4	75.0	81.8	81.8	85.7	.....	.....
	5	A	.2	1.2	1.3	1.8	2.4	1.8	1.8	1.7	1.2	0.7	.....	80.0	81.3	80.3	79.3	78.0	80.0	80.9	80.5	80.6	78.8
		B	.2	1.6	1.9	2.2	2.6	2.6	2.5	1.8	1.6	.....	.....	80.0	78.4	77.9	79.2	77.6	77.2	79.2	78.6	79.7	.....
	6	A	.2	2.2	3.6	3.7	4.1	3.8	3.0	2.0	1.9	.....	.....	90.9	79.2	78.6	77.4	78.4	78.9	80.8	76.7	77.4	.....
		B	.3	2.8	3.4	4.0	4.0	3.8	3.6	.....	.....	.....	.....	62.5	78.3	77.9	78.8	78.7	79.7	77.8	.....	.....	.....
	7	A	.1	3.7	3.7	5.3	5.6	5.6	5.1	4.2	4.1	.....	.....	80.0	79.4	79.9	78.4	78.1	78.3	78.6	79.6	78.5	.....
		B	2.7	4.0	3.9	4.4	5.1	5.1	5.0	4.3	3.9	3.0	.....	81.3	80.0	79.9	80.3	79.8	79.8	79.7	73.3	79.2	78.6
	8	A	.5	4.7	6.1	6.9	6.7	6.6	5.3	4.8	.....	.....	.....	86.1	79.7	79.0	79.2	79.3	79.2	79.6	79.8	.....	.....
		B	3.9	5.1	4.9	6.4	6.3	6.2	4.8	3.9	.....	.....	.....	79.7	79.3	79.8	78.9	79.1	78.9	79.8	79.4	.....	.....
	9	A	3.5	5.8	7.6	5.5	.....	.....	.....	.....	.....	.....	.....	79.5	79.1	77.4	78.7	.....	.....	.....	.....	.....	.....
		B	3.7	5.9	6.9	7.9	7.7	7.2	5.9	3.4	.....	.....	.....	79.2	79.2	78.8	78.2	77.9	78.4	78.6	79.3	.....	.....
	10	A	4.8	5.8	8.2	8.8	7.8	7.7	6.8	5.1	.....	.....	.....	78.0	78.7	77.6	77.3	77.7	77.4	76.4	77.8	.....	.....
		B	7.7	9.8	12.5	12.7	11.6	12.9	13.0	12.2	10.5	7.5	.....	77.6	76.6	74.7	74.5	74.8	74.6	73.7	74.0	74.3	75.6
	11	A	8.9	10.4	12.4	13.1	12.9	11.3	10.7	9.8	7.3	.....	.....	76.7	76.9	75.7	76.0	76.0	77.4	76.7	77.0	81.3	.....
		B	10.3	11.6	11.1	11.7	11.9	10.9	7.4	.....	.....	.....	.....	76.2	76.0	76.4	76.8	76.5	75.5	75.3	.....	.....	.....
	12	A	11.5	12.7	13.9	13.4	13.0	11.4	.....	.....	.....	.....	.....	74.9	74.3	72.5	72.9	73.5	74.0	.....	.....	.....	.....
		B	8.1	10.8	11.7	12.5	11.5	10.1	6.4	.....	.....	.....	.....	74.4	73.8	74.5	74.2	75.0	75.0	74.4	.....	.....	.....
	13	A	10.6	15.1	13.8	12.5	11.7	.....	.....	.....	.....	.....	.....	74.6	74.2	74.1	74.3	73.8	.....	.....	.....	.....	.....
		B	11.0	13.1	13.7	13.0	7.8	9.2	8.2	.....	.....	.....	.....	74.8	74.5	75.4	76.1	75.5	75.8	76.6	.....	.....	.....
	14	A	12.7	16.6	18.9	18.8	21.7	20.4	19.5	15.2	.....	.....	.....	73.1	73.4	71.2	70.9	69.5	71.9	71.9	67.4	.....	.....
		B	18.4	16.5	16.4	18.7	16.2	19.2	16.1	16.4	14.5	.....	.....	75.0	72.8	72.1	71.4	72.7	71.1	73.8	73.1	72.5	.....
	15	A	17.5	20.4	20.5	22.1	21.2	22.2	19.8	19.1	15.0	.....	.....	72.0	72.2	72.6	71.1	70.4	71.3	71.1	72.3	71.3	.....
		B	19.4	24.2	25.7	27.3	26.8	26.1	28.5	24.5	20.6	.....	.....	71.7	70.9	70.0	70.1	68.2	69.5	67.7	68.8	70.2	.....
	16	A	18.8	22.4	23.4	23.7	18.8	.....	.....	.....	.....	.....	.....	69.7	64.3	68.2	68.2	68.1	.....	.....	.....	.....	.....
		B	16.9	23.0	24.2	25.0	23.4	22.5	19.6	.....	.....	.....	.....	71.0	71.2	70.2	70.1	69.5	69.4	69.0	.....	.....	.....
		C	16.8	22.1	23.5	24.7	25.6	22.7	20.2	.....	.....	.....	.....	69.8	70.7	69.9	69.4	68.4	68.0	68.5	.....	.....	.....
	17	A	18.0	22.8	23.2	24.4	23.3	19.6	.....	.....	.....	.....	.....	69.6	69.9	69.5	68.8	69.7	67.5	.....	.....	.....	.....
		B	21.3	25.0	25.6	26.1	24.0	20.4	.....	.....	.....	.....	.....	69.7	70.0	70.7	70.5	69.4	68.6	.....	.....	.....	.....
		C	24.9	26.0	23.1	23.1	20.4	.....	.....	.....	.....	.....	.....	68.5	66.7	65.7	64.9	68.6	.....	.....	.....	.....	.....
	18	A	23.4	27.1	26.4	25.7	23.2	.....	.....	.....	.....	.....	.....	69.2	66.3	69.0	68.6	69.1	.....	.....	.....	.....	.....
		B	19.4	29.6	30.2	31.7	32.0	31.8	28.7	25.5	.....	.....	.....	66.7	67.9	68.4	67.1	65.8	65.1	66.1	67.6	.....	.....
		C	21.3	25.7	25.6	25.2	23.7	18.4	.....	.....	.....	.....	.....	69.6	67.5	68.7	67.4	67.3	65.8	.....	.....	.....	.....
	19	A	12.3	27.0	27.1	22.4	21.3	.....	.....	.....	.....	.....	.....	68.4	68.2	67.2	65.6	66.0	.....	.....	.....	.....	.....
		B	22.5	27.8	29.2	27.9	25.2	.....	.....	.....	.....	.....	.....	68.1	67.6	65.8	66.3	67.5	.....	.....	.....	.....	.....
		C	17.0	23.0	25.1	24.4	23.7	22.3	22.6	21.4	.....	.....	.....	66.8	66.2	65.2	64.4	64.6	65.6	64.4	63.0	.....	.....

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*—Continued

SECTION 2: BAKU, 1919—continued.

Date.	Spike.	Dry weight of kernels (mgm.).											Water in kernels (per cent).										
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
July 20	A	30.2	31.2	27.0	.....	.....	.....	.....	.....	.....	.....	.....	64.0	64.2	65.6	.....	.....	.....	.....	.....	.....	.....	.....
	B	11.8	28.6	29.1	28.6	29.2	20.2	.....	.....	.....	.....	.....	62.4	63.3	63.5	62.8	55.7	.....	.....	.....	.....	.....	.....
	C	21.4	30.5	33.4	34.4	35.2	33.0	32.8	31.0	.....	.....	.....	65.0	65.0	64.6	64.6	60.2	61.6	60.6	62.2	.....	.....	.....
21	A	30.6	32.0	26.7	31.3	22.2	.....	.....	.....	.....	.....	.....	67.0	62.2	65.2	62.7	65.0	.....	.....	.....	.....	.....	.....
	B	23.2	28.0	32.2	34.6	35.9	33.6	33.3	26.5	.....	.....	.....	63.4	63.6	63.2	60.8	62.2	57.1	57.1	56.6	.....	.....	.....
	C	27.0	34.2	36.2	32.9	34.6	33.1	30.3	27.7	.....	.....	.....	67.1	65.2	62.9	64.0	59.7	63.7	62.4	61.8	.....	.....	.....
	A	17.4	23.9	23.3	22.5	18.5	.....	.....	.....	.....	.....	.....	64.2	60.8	.....	61.6	62.1	61.1	.....	.....	.....	.....	.....
22	B	33.5	36.8	38.0	40.0	39.7	33.6	33.8	.....	.....	.....	.....	65.2	67.9	65.3	63.2	60.7	60.3	57.9	.....	.....	.....	.....
	C	29.9	34.7	32.9	30.1	19.3	.....	.....	.....	.....	.....	.....	65.9	61.6	65.2	63.9	67.7	.....	.....	.....	.....	.....	.....
	D	30.1	28.3	32.3	34.2	35.2	26.9	.....	.....	.....	.....	.....	62.8	63.5	63.7	53.7	58.8	61.8	.....	.....	.....	.....	.....
	E	23.8	27.6	29.2	29.3	30.0	27.2	.....	.....	.....	.....	.....	62.0	58.9	45.9	56.5	55.2	49.6	.....	.....	.....	.....	.....
	A	27.2	28.7	30.3	30.3	29.1	19.5	.....	.....	.....	.....	.....	63.4	63.7	62.3	61.2	61.5	58.9	.....	.....	.....	.....	.....
23	B	25.8	34.0	32.8	36.6	36.4	33.4	26.6	.....	.....	.....	.....	63.1	62.0	59.7	59.5	57.8	57.5	53.5	.....	.....	.....	.....
	C	29.9	30.3	30.2	29.6	21.4	.....	.....	.....	.....	.....	.....	63.8	62.0	60.4	62.7	60.5	.....	.....	.....	.....	.....	.....
	D	27.2	36.0	40.3	35.5	36.6	34.3	26.9	.....	.....	.....	.....	62.1	59.0	59.0	59.8	58.0	56.3	55.6	.....	.....	.....	.....
	E	21.0	28.1	29.8	29.4	22.9	.....	.....	.....	.....	.....	.....	60.7	60.8	62.0	59.7	58.4	.....	.....	.....	.....	.....	.....
	A	31.1	37.9	39.5	41.7	44.3	42.7	41.7	35.1	.....	.....	.....	61.5	61.3	60.2	59.4	56.8	56.0	53.7	50.7	.....	.....	.....
	B	28.3	38.1	38.8	37.6	36.7	36.0	32.3	27.1	.....	.....	.....	61.6	59.1	56.6	48.6	41.7	49.6	49.1	41.6	.....	.....	.....
24	C	30.3	40.6	40.0	28.6	32.0	33.0	.....	.....	.....	.....	.....	63.8	59.8	62.2	64.3	63.3	60.7	.....	.....	.....	.....	.....
	D	36.4	36.2	32.4	26.1	.....	.....	.....	.....	.....	.....	.....	61.8	60.7	64.7	57.8	.....	.....	.....	.....	.....	.....	.....
	E	32.6	39.5	38.3	31.6	34.2	32.6	.....	.....	.....	.....	.....	61.4	59.7	60.7	58.9	59.3	60.7	.....	.....	.....	.....	.....
	A	32.3	34.1	33.4	34.1	27.0	.....	.....	.....	.....	.....	.....	61.6	60.9	59.7	59.3	60.5	.....	.....	.....	.....	.....	.....
	B	26.5	38.4	42.4	46.1	45.9	45.7	41.1	30.3	.....	.....	.....	62.7	61.9	61.7	59.8	55.9	54.4	52.4	51.4	.....	.....	.....
25	C	30.0	38.6	40.1	40.4	46.3	40.6	24.3	.....	.....	.....	.....	63.2	57.5	58.6	35.0	52.5	47.1	17.3	.....	.....	.....	.....
	D	36.1	32.9	34.9	29.6	26.5	.....	.....	.....	.....	.....	.....	60.4	60.0	57.9	59.2	54.1	.....	.....	.....	.....	.....	.....
	E	32.2	41.1	41.5	40.3	31.9	29.6	.....	.....	.....	.....	.....	61.7	57.9	58.0	56.9	56.1	56.4	.....	.....	.....	.....	.....
	A	20.6	29.4	29.4	30.6	30.5	29.1	31.0	27.1	10.5	.....	.....	57.9	59.8	58.9	57.1	48.7	43.2	40.6	18.9	57.1	.....	.....
	B	24.2	32.1	33.1	34.6	28.4	35.9	35.9	33.8	29.9	28.9	.....	55.0	17.9	45.0	54.0	12.3	46.0	45.3	40.2	26.2	16.7	.....
26	C	42.6	49.5	48.4	48.9	45.0	43.6	40.3	40.4	36.2	.....	.....	27.3	41.4	43.5	51.1	23.6	27.7	15.0	15.1	13.0	.....	.....
	D	38.0	38.6	37.7	38.4	34.1	30.4	18.8	.....	.....	.....	.....	51.7	48.9	43.1	48.8	24.8	16.9	51.2	.....	.....	.....	.....
	E	27.9	45.3	47.6	47.7	49.9	46.7	47.2	39.7	.....	.....	.....	59.3	61.0	57.5	57.1	52.9	53.0	50.0	45.9	.....	.....	.....
	A	39.1	36.7	38.9	39.4	35.1	25.5	.....	.....	.....	.....	.....	52.1	16.4	28.1	24.8	8.6	10.2	.....	.....	.....	.....	.....
27	B	27.8	26.3	40.7	41.3	40.0	39.0	32.7	29.7	.....	.....	.....	55.3	57.9	53.6	52.5	50.2	43.8	14.6	10.8	.....	.....	.....
	C	26.2	41.8	46.5	44.0	45.1	42.4	29.0	.....	.....	.....	.....	59.1	53.5	50.2	44.9	32.1	46.3	12.9	.....	.....	.....	.....
	D	40.6	42.7	42.0	40.8	36.3	.....	.....	.....	.....	.....	.....	54.2	56.1	53.0	48.9	47.0	.....	.....	.....	.....	.....	.....
	E	40.5	47.0	48.5	47.9	49.7	44.3	39.4	28.3	.....	.....	.....	57.1	55.5	53.4	47.8	45.7	46.5	39.6	15.3	.....	.....	.....
	A	33.9	38.1	36.7	36.6	33.0	33.4	26.6	.....	.....	.....	.....	49.2	39.0	28.2	29.3	10.3	8.0	8.3	.....	.....	.....	.....
28	B	27.9	35.7	35.0	34.8	35.5	28.3	.....	.....	.....	.....	.....	60.3	23.2	33.2	46.9	16.3	11.0	.....	.....	.....	.....	.....
	C	25.6	35.8	37.8	39.1	37.0	30.5	24.9	.....	.....	.....	.....	52.9	52.3	50.8	51.4	44.8	22.6	11.4	.....	.....	.....	.....
	D	37.2	36.3	44.7	41.8	42.3	37.9	31.4	.....	.....	.....	.....	55.3	53.1	52.2	42.5	29.5	37.3	15.4	.....	.....	.....	.....
	E	24.6	33.2	33.2	34.0	32.9	29.4	20.9	.....	.....	.....	.....	49.9	49.2	45.7	18.5	12.0	10.4	10.7	.....	.....	.....	.....

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TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*—Continued

SECTION 3: BAKU, 1920.

Date.	Spike.	Dry weight of kernels (mgm.).											Water in kernels (per cent).										
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
July 10	A	0.6	.....	1.7	2.0	2.3	2.2	2.1	2.0	2.0	1.7	1.0	80.0	.....	80.2	79.2	79.3	79.6	80.7	80.6	79.6	78.7	77.8
	B	.5	0.7	1.4	1.6	1.9	2.1	2.2	1.8	1.9	1.7	1.1	80.8	84.1	80.0	81.4	81.0	80.9	80.2	81.8	80.6	81.1	82.8
	C	.4	.4	.4	.7	.6	.6	.6	.6	.5	.3	.....	77.8	81.8	86.2	82.9	84.2	83.8	83.3	81.8	83.3	83.3	.....
11	A	1.9	3.3	3.8	4.4	4.6	4.8	4.5	4.3	4.1	3.5	3.0	81.7	80.6	80.3	79.8	79.0	79.7	78.9	79.4	79.5	79.2	79.0
	B	1.6	2.1	2.6	2.7	3.1	2.7	2.5	2.7	2.1	2.0	.....	81.4	81.2	81.3	80.1	80.2	80.7	80.5	80.4	80.7	80.0	.....
	C	.7	.9	1.4	1.6	1.7	1.7	1.7	1.7	1.6	1.3	.....	80.0	80.4	80.6	79.7	79.3	79.8	79.3	79.8	80.0	79.7	.....
12	A	2.4	3.8	4.8	5.4	5.7	5.5	5.2	5.1	4.1	3.2	.....	80.9	79.8	79.3	79.2	78.5	78.7	78.9	78.9	79.4	78.9	.....
	B	3.0	3.6	4.2	4.3	4.6	4.3	4.2	3.7	3.5	.....	.....	80.5	80.4	80.3	79.9	79.6	79.9	80.1	79.9	79.4	.....	.....
	C	1.3	1.7	2.3	2.6	2.6	2.6	2.6	2.5	2.2	1.4	.....	81.9	80.5	80.5	79.8	80.6	80.6	80.0	80.0	80.0	80.0	.....
13	A	4.1	5.1	5.2	5.6	5.5	6.0	5.5	5.1	4.3	.....	.....	80.3	79.3	78.9	78.7	78.8	78.3	78.6	78.9	78.3	.....	.....
	B	3.1	3.9	4.4	4.7	4.7	4.4	4.7	3.5	.....	.....	.....	80.0	80.3	78.8	79.0	79.0	78.4	78.2	79.2	.....	.....	.....
	C	1.1	1.8	2.8	3.2	3.4	3.4	3.2	3.4	3.0	2.8	1.8	83.3	82.3	81.3	80.2	80.6	80.9	80.8	80.1	81.1	81.1	80.8
14	A	4.6	6.4	7.2	8.4	9.5	9.4	8.2	8.1	7.3	6.6	.....	79.6	77.8	78.0	77.3	76.7	76.7	77.5	77.4	77.8	78.4	.....
	B	3.7	5.8	7.7	8.1	8.4	8.9	9.0	7.4	7.1	5.7	.....	79.9	79.0	78.2	77.7	77.6	77.1	77.5	78.2	78.2	78.1	.....
	C	3.0	3.4	4.1	4.4	4.4	4.3	4.4	4.0	3.5	2.8	.....	81.0	81.3	80.1	79.4	79.9	79.7	79.6	79.8	80.1	79.7	.....
15	A	8.4	10.2	11.3	11.8	12.1	11.9	11.9	11.6	9.5	7.9	.....	76.8	76.3	75.4	75.4	75.5	75.1	75.2	75.0	75.5	75.5	.....
	B	5.8	7.4	8.4	9.6	9.8	9.8	9.8	9.8	9.2	8.2	7.6	79.8	78.2	77.1	76.3	76.6	77.3	75.9	75.8	75.6	76.2	76.4
	C	2.6	3.5	4.4	4.5	5.0	5.4	5.4	5.2	4.7	4.2	.....	80.4	80.1	79.2	78.6	78.3	78.4	78.6	78.1	78.4	77.5	.....
16	A	9.5	11.9	13.3	14.6	14.8	14.3	13.4	13.7	12.1	9.7	.....	76.7	75.6	74.7	73.2	73.4	74.1	74.9	74.7	74.7	74.0	.....
	B	5.1	8.2	9.9	10.9	12.2	12.5	12.0	11.6	11.2	9.8	.....	78.8	77.5	76.3	75.7	75.2	75.3	75.3	75.3	76.3	76.2	.....
	C	3.5	5.8	6.3	7.7	8.0	7.9	7.8	7.8	7.4	6.6	5.0	79.4	78.6	78.3	77.4	77.5	77.5	77.9	77.6	78.0	78.1	77.9
17	A	9.6	16.5	17.0	19.4	19.2	19.8	19.9	19.1	18.9	17.4	15.5	76.0	74.3	73.9	73.8	73.9	73.4	73.3	73.3	73.4	73.3	73.6
	B	9.1	12.3	14.1	14.6	15.1	14.8	14.4	12.8	10.4	.....	.....	77.1	75.8	75.2	74.7	74.7	74.7	74.7	75.2	74.8	.....	.....
	C	5.4	7.6	8.7	9.1	8.7	9.6	9.5	9.2	8.9	8.3	7.2	78.7	77.4	77.1	77.1	76.7	76.5	77.2	76.8	77.2	77.1	77.1
18	A	13.5	15.8	19.4	18.8	17.3	19.5	19.4	17.1	16.8	16.4	12.6	74.7	74.2	73.1	72.8	73.3	71.9	71.8	72.5	71.8	72.2	71.6
	B	10.7	13.6	15.1	16.8	18.4	15.9	11.0	.....	.....	.....	.....	76.8	75.8	74.8	75.1	73.7	68.0	74.3	.....	.....	.....	.....
	C	9.1	10.6	11.1	12.4	12.8	13.2	13.1	13.1	13.2	12.0	11.3	76.6	75.0	75.1	75.0	74.7	74.9	74.6	75.3	75.0	74.9	75.4
19	A	11.8	14.9	18.8	20.1	18.4	19.7	18.1	16.5	13.4	.....	.....	74.8	73.9	72.1	71.8	72.4	71.9	71.7	72.4	71.7	.....	.....
	B	12.1	18.8	20.8	22.8	22.8	22.3	20.0	16.6	.....	.....	.....	76.4	73.9	73.1	73.0	72.9	73.2	73.1	73.7	.....	.....	.....
	C	9.0	12.8	14.4	16.3	17.2	17.4	17.4	16.9	15.8	14.3	.....	76.0	75.5	74.7	74.1	73.9	74.1	73.9	73.8	74.0	74.1	.....
20	A	14.1	23.9	26.7	27.0	26.8	28.0	27.0	25.1	24.4	21.2	.....	72.9	66.7	70.2	70.9	70.2	70.3	69.8	70.6	69.0	70.0	.....
	B	9.1	18.3	19.9	23.5	24.8	24.7	24.0	24.0	23.5	21.4	.....	74.8	72.3	70.9	69.8	70.5	69.8	70.0	69.5	69.4	67.9	.....
	C	6.7	14.5	17.3	17.8	18.4	17.1	17.6	16.9	16.2	14.6	.....	74.1	71.7	70.3	69.9	70.6	69.3	69.9	69.8	70.0	71.1	.....
21	A	23.5	29.8	29.5	30.6	32.2	30.0	29.2	28.0	26.7	24.7	.....	69.8	67.5	68.8	67.5	67.6	66.9	67.9	68.0	67.7	66.9	.....
	B	16.4	23.7	26.1	26.4	27.3	26.7	26.3	25.4	22.8	14.7	.....	72.0	70.6	69.5	69.7	68.9	69.8	68.6	69.5	69.9	69.5	.....
	C	18.0	21.1	21.5	23.1	24.7	23.4	23.3	22.5	21.8	19.6	16.3	72.8	71.9	71.8	71.3	71.0	71.9	71.9	72.2	71.8	72.2	72.1
22	A	23.4	32.3	34.0	33.1	33.9	33.0	32.3	30.0	24.1	.....	.....	67.2	68.1	66.4	66.5	67.1	66.2	66.3	65.8	64.9	.....	.....
	B	22.4	24.9	25.2	27.2	27.1	29.2	29.8	27.8	25.2	25.8	20.8	68.1	67.9	66.1	66.6	66.7	66.6	66.4	65.9	66.1	66.4	66.4
	C	19.7	22.3	23.7	25.8	26.9	26.0	26.0	24.9	23.7	20.2	.....	70.8	70.7	70.4	69.7	69.1	69.0	69.1	69.4	69.2	68.7	.....
23	A	30.0	34.5	35.8	37.7	38.5	37.3	36.4	33.0	32.2	25.8	.....	68.2	66.9	66.3	66.0	65.5	65.1	65.1	64.1	63.8	61.4	.....
	B	29.3	33.0	33.6	35.3	34.3	34.4	33.0	30.9	27.5	.....	.....	67.0	66.3	64.7	66.1	64.3	63.4	64.0	64.3	64.4	.....	.....
	C	17.6	24.9	26.9	26.5	26.8	27.8	28.5	27.0	26.2	23.0	.....	70.4	69.5	68.3	68.4	69.0	68.2	68.0	68.6	68.3	67.8	.....

24	A	34.2	38.1	39.8	40.6	38.8	37.7	35.4	32.3	.....	.....	.....	65.0	65.6	65.5	64.0	63.9	64.3	64.4	63.8	.....	.....	.....
	B	20.2	27.0	28.7	30.4	29.1	29.6	27.8	27.2	25.6	.....	.....	67.4	65.7	66.1	65.1	64.5	63.9	63.9	64.2	63.5	.....	.....
	C	24.3	29.7	30.2	31.3	32.4	30.7	30.4	29.1	25.8	.....	.....	71.5	69.5	69.4	69.0	68.9	68.3	68.5	68.6	68.1	.....	.....
25	A	30.9	40.4	43.5	42.9	43.2	44.6	42.2	40.7	40.2	36.4	33.8	63.0	64.2	62.9	62.5	61.8	61.1	60.9	60.4	59.9	60.1	58.3
	B	31.9	34.4	37.6	38.8	38.4	37.1	37.3	35.5	33.1	31.1	23.9	63.7	64.0	62.2	62.7	63.0	62.4	61.9	62.5	61.7	61.2	60.2
	C	18.5	27.3	29.6	30.8	30.8	32.2	33.3	32.4	33.3	32.2	29.5	69.3	68.3	68.4	68.4	68.3	67.1	66.2	66.1	66.4	66.2	65.0
	D	21.0	30.5	32.9	34.5	34.1	33.4	32.9	31.6	30.7	.....	.....	66.9	67.8	67.1	66.6	66.2	65.3	64.9	64.6	68.2	.....	.....
	E	36.8	40.1	41.2	41.7	40.6	40.3	39.0	36.9	35.7	30.0	.....	67.3	65.4	64.5	63.8	63.0	63.0	63.0	62.7	62.4	60.9	.....
26	A	31.3	34.9	35.7	38.4	38.0	36.4	35.9	33.6	32.7	31.7	28.1	62.1	61.5	61.2	62.2	60.9	61.0	60.7	61.9	61.0	59.2	60.7
	B	19.9	35.4	39.3	40.5	42.7	41.4	38.2	36.3	34.8	.....	.....	61.5	62.9	63.4	61.2	60.6	61.1	59.7	60.0	56.4	.....	.....
	C	26.6	32.3	33.6	36.2	37.9	35.9	34.7	37.1	33.5	32.5	27.4	64.7	64.8	64.1	63.8	62.5	63.6	62.2	58.4	62.4	62.2	61.1
	D	29.5	35.0	35.8	37.2	36.9	37.2	37.5	34.8	34.9	32.8	.....	65.7	65.8	65.7	65.6	63.8	63.3	63.3	64.2	63.0	62.2	.....
	E	27.6	30.0	31.1	30.6	30.3	30.7	29.9	28.4	27.9	.....	.....	67.1	64.3	64.4	63.8	63.8	64.6	63.8	63.4	64.1	.....	.....
27	A	27.2	36.7	39.8	37.5	39.4	40.6	30.3	35.4	35.0	.....	.....	64.3	63.1	63.4	63.4	61.7	60.2	59.8	61.6	59.2	.....	.....
	B	35.1	38.0	39.4	41.0	39.8	39.9	38.7	36.6	31.8	.....	.....	63.0	63.9	63.4	61.6	61.6	60.5	60.8	61.1	59.8	.....	.....
	C	29.4	35.2	37.0	39.2	38.5	38.3	37.5	36.2	34.5	32.0	.....	66.1	65.5	64.7	62.8	63.2	63.0	62.6	62.4	62.2	62.3	.....
	D	30.0	33.3	34.9	33.6	33.4	32.3	32.5	31.8	.....	.....	.....	64.9	64.6	61.8	62.0	62.6	62.3	62.1	61.6	.....	.....	.....
	E	29.3	33.8	33.0	34.2	34.3	33.3	33.2	32.9	31.2	29.4	.....	66.8	65.9	65.4	63.1	62.5	62.1	62.1	61.7	61.5	60.5	.....
28	A	29.5	41.5	45.0	43.4	44.7	45.8	44.7	42.8	43.1	41.1	37.5	61.4	61.4	60.9	60.7	60.4	58.7	57.6	57.2	57.0	56.2	55.7
	B	37.9	41.6	46.0	45.6	45.4	44.6	42.0	41.3	36.8	.....	.....	61.5	61.9	60.6	59.6	60.2	58.2	58.2	57.2	57.4	.....	.....
	C	36.6	43.3	43.8	43.3	43.8	42.7	41.0	39.9	36.8	.....	.....	65.7	63.5	63.2	62.9	61.8	61.9	61.6	61.6	60.9	.....	.....
	D	29.5	38.8	42.9	44.7	42.6	43.3	41.8	41.8	41.0	49.2	34.5	63.8	63.1	63.1	62.1	62.7	62.0	61.3	60.8	59.8	50.5	60.9
	E	34.2	34.8	37.4	37.1	39.1	36.9	32.6	.....	.....	.....	.....	64.4	62.9	62.8	62.3	59.3	61.3	61.2	.....	.....	.....	.....
29	A	39.7	48.0	46.8	45.4	43.6	43.1	41.0	36.2	.....	.....	.....	62.8	60.7	59.1	58.9	59.5	58.6	56.8	57.1	.....	.....	.....
	B	40.0	44.8	45.5	45.9	46.2	44.8	44.4	43.3	40.3	30.9	.....	60.7	60.4	59.0	57.9	58.1	56.8	56.2	56.7	56.0	55.7	.....
	C	34.6	38.0	39.7	38.3	40.0	39.9	39.1	37.6	36.5	23.7	.....	63.7	62.0	62.0	61.0	61.1	61.3	60.3	60.2	59.9	58.4	.....
	D	26.9	38.4	40.6	42.8	42.2	41.3	40.9	40.3	38.8	31.5	.....	63.1	62.7	62.2	61.8	62.0	60.5	60.5	59.4	59.3	59.1	.....
	E	29.5	35.7	36.4	36.8	35.3	35.7	36.9	37.1	34.6	32.9	29.1	64.2	63.3	63.6	62.6	62.3	63.3	62.1	62.7	62.5	60.8	61.4
30	A	40.4	45.8	48.8	47.6	49.6	50.1	48.4	47.1	43.9	41.1	.....	57.4	57.5	56.3	56.0	55.9	54.3	55.1	54.0	53.5	53.2	.....
	B	41.3	47.0	50.5	50.2	49.1	47.9	48.3	44.0	42.9	.....	.....	59.6	59.7	58.3	59.1	57.9	57.2	56.6	56.4	55.4	.....	.....
	C	35.2	45.0	45.8	46.8	47.5	45.7	45.0	44.0	41.7	46.4	.....	61.9	61.3	62.0	61.4	61.4	60.3	60.0	60.0	59.7	46.4	.....
	D	38.7	40.8	43.0	41.2	40.6	41.8	39.4	37.4	36.1	.....	.....	62.2	61.4	61.3	60.2	60.7	59.8	58.7	58.5	58.9	.....	.....
	E	40.3	43.4	44.4	44.1	45.0	43.6	43.0	42.0	40.3	45.6	.....	62.6	62.6	61.2	61.5	59.8	60.3	59.7	58.9	59.3	45.8	.....
31	A	40.1	49.1	48.8	50.8	50.7	49.9	48.9	46.0	44.9	42.0	.....	58.4	57.7	56.6	55.4	54.9	54.2	54.2	53.8	53.4	53.2	.....
	B	45.1	48.5	48.1	48.7	47.3	46.2	44.8	44.5	43.4	40.8	37.4	58.2	58.2	57.3	56.6	55.1	55.0	55.2	54.5	53.5	54.0	53.4
	C	.....	41.3	44.5	44.8	42.5	41.2	37.7	35.8	36.8	31.7	.....	.....	60.1	60.2	59.5	59.4	58.3	59.0	58.8	58.2	57.4	.....
	D	31.7	42.3	44.1	46.0	46.9	46.2	44.6	42.0	42.5	35.8	.....	60.3	58.1	57.5	56.8	56.4	56.0	55.4	55.1	55.1	54.7	.....
	E	34.0	42.0	37.8	38.4	38.4	41.3	39.1	37.9	38.7	35.2	34.5	59.5	59.9	59.3	56.5	58.7	55.2	54.0	59.4	58.4	57.0	54.7
Aug. 1	A	37.6	52.8	55.8	58.2	56.3	57.7	55.4	52.2	53.3	49.7	47.1	54.3	55.4	56.0	54.0	55.0	53.2	53.2	53.2	52.0	51.7	51.3
	B	45.0	50.1	51.6	53.5	53.0	50.3	48.5	47.7	41.8	.....	.....	54.0	55.0	53.0	54.3	52.9	51.6	51.3	51.1	50.0	.....	.....
	C	29.7	39.0	41.9	42.5	43.1	44.3	43.5	39.5	41.7	38.9	33.4	60.0	61.2	59.9	58.5	57.9	57.3	56.8	58.0	55.9	55.1	55.7
	D	30.0	41.0	45.1	47.1	45.3	31.0	44.1	44.4	38.4	34.3	.....	59.0	58.5	57.8	56.3	55.7	59.9	53.3	53.8	53.7	54.2	.....
	E	40.5	46.0	47.7	49.3	47.8	46.8	46.5	45.5	42.1	35.4	.....	55.7	54.5	54.7	53.8	53.6	52.8	53.2	52.7	52.2	51.5	.....
2	A	45.6	53.3	54.4	55.6	56.1	54.6	53.4	51.8	49.6	44.4	37.4	53.7	53.7	53.9	53.3	52.7	51.9	51.0	51.0	50.7	50.3	47.2
	B	37.1	43.6	48.8	49.9	50.0	48.2	45.5	45.6	41.8	33.7	.....	54.2	54.2	54.0	52.0	52.1	51.1	49.5	50.5	49.9	49.9	.....
	C	34.1	41.4	44.4	43.1	45.8	43.0	42.6	40.4	48.6	.....	.....	54.5	54.9	54.1	53.6	40.9	52.2	51.9	51.6	38.3	.....	.....
	D	28.5	43.7	46.8	47.0	48.5	45.3	45.6	.....	48.2	.....	.....	59.7	57.6	58.5	57.3	55.8	55.7	54.8	.....	43.1	.....	.....
	E	32.5	46.8	49.6	50.7	50.2	49.4	48.3	45.0	.....	.....	.....	57.6	57.5	57.2	56.4	55.8	55.7	55.3	55.0	.....	.....	.....
3	A	49.4	57.3	59.1	60.0	57.0	57.2	55.3	52.6	49.4	.....	.....	54.2	54.3	52.9	52.3	52.7	51.6	51.7	50.7	47.8	.....	.....
	B	31.0	39.9	40.8	41.9	43.1	41.8	41.4	41.6	39.8	37.4	34.0	49.4	47.6	53.7	51.6	46.5	53.0	43.8	44.4	41.6	38.3	20.6
	C	44.5	50.2	50.8	50.3	50.4	49.7	49.8	46.8	43.9	43.3	.....	56.7	56.5	55.2	54.9	54.1	54.4	53.8	53.4	53.1	52.0	.....
	D	.....	45.9	45.8	46.8	47.2	46.6	48.4	48.2	48.3	.....	.....	54.1	54.2	53.2	52.8	53.4	51.6	51.8	51.7	.....	.....	.....
	E	42.4	51.0	52.4	54.4	52.0	52.2	50.4	46.3	36.2	.....	.....	54.3	54.6	54.8	53.2	52.8	52.8	52.4	52.3	50.9	.....	.....

TABLE 1.—*Dry weight and percentage of water in individual kernels of barley from flowering to maturity at Aberdeen, Idaho*—Continued

SECTION 3: BAKU, 1920—Continued

Date.	Spike.	Dry weight of kernels (mgm.).											Water in kernels (per cent).										
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Aug. 4	A	44.2	52.6	56.1	56.9	56.9	55.7	52.3	52.0	48.3	.....	.....	51.5	51.3	52.6	51.7	50.8	50.0	49.1	49.0	48.3	.....	.....
	B	41.1	49.7	54.2	55.6	54.6	54.1	52.3	51.1	48.7	44.2	.....	57.2	52.9	52.9	52.9	51.2	51.6	49.1	48.9	46.8	28.2	.....
	C	48.5	50.7	49.2	48.7	50.1	47.7	47.0	45.8	43.8	38.4	31.7	55.2	54.6	52.6	53.3	53.2	53.9	52.4	53.9	52.1	52.0	47.1
	D	31.8	44.8	44.6	47.7	47.4	46.8	44.4	43.9	42.2	35.1	24.5	56.3	54.2	55.0	53.9	53.8	51.9	52.9	50.1	50.8	46.1	23.9
	E	32.6	34.0	36.0	35.5	37.1	32.7	34.9	32.7	34.2	29.2	28.1	53.8	46.5	48.1	32.2	41.8	22.5	32.4	25.8	33.7	19.3	18.3
5	A	42.5	50.3	53.6	52.6	53.7	52.3	50.7	49.7	45.0	44.5	44.3	48.9	50.8	48.2	44.0	45.1	45.5	44.1	47.2	45.1	29.0	31.2
	B	35.8	44.8	47.8	50.9	48.4	47.2	46.3	44.8	41.2	39.3	.....	56.1	55.6	54.7	53.0	52.5	52.0	50.9	52.0	48.8	43.5	.....
	C	46.0	54.6	55.3	57.7	57.7	55.8	54.0	52.0	50.8	42.2	.....	53.5	54.5	54.1	52.7	52.5	52.0	51.8	51.4	51.0	49.8	.....
	D	43.4	47.6	47.6	48.0	44.5	45.0	43.0	38.7	36.1	.....	.....	56.7	55.1	54.1	53.3	49.0	47.4	43.9	30.3	31.2	.....	.....
	E	29.1	40.2	41.2	39.8	37.3	34.9	31.8	26.0	.....	.....	.....	48.7	50.1	49.6	45.5	40.2	28.3	20.1	17.5	.....	.....	.....
6	A	48.4	56.7	60.8	61.4	59.3	57.0	54.7	42.1	49.0	.....	.....	54.3	50.0	50.3	49.3	47.5	43.3	39.6	41.5	36.2	.....	.....
	B	39.5	52.0	54.4	55.4	54.9	53.6	50.8	48.0	45.6	39.5	.....	50.9	50.2	49.2	49.4	46.0	38.6	33.1	26.8	20.4	17.4	.....
	C	42.7	45.8	44.5	42.7	43.6	40.9	37.2	32.4	.....	.....	.....	48.7	50.1	50.1	48.2	47.3	42.6	33.1	25.3	.....	.....	.....
	D	48.0	56.1	56.5	56.6	57.2	55.7	53.1	55.6	53.9	50.0	44.3	51.9	53.2	52.7	53.8	52.9	51.9	53.7	50.4	47.3	48.4	41.7
	E	20.4	38.3	35.4	40.8	41.8	36.1	38.1	38.1	35.0	36.3	29.5	63.4	41.1	45.8	38.3	38.7	31.2	27.4	26.2	25.2	22.4	19.0
7	A	51.5	58.6	59.9	61.7	60.8	59.2	56.1	55.6	55.1	50.2	38.8	47.3	48.0	46.2	46.2	46.5	44.6	42.5	43.5	39.0	31.8	23.3
	B	37.7	57.6	56.6	59.7	59.8	58.4	56.7	54.6	50.6	48.7	.....	48.2	49.9	50.1	49.9	49.4	48.3	48.3	43.5	37.1	31.0	.....
	C	32.8	46.5	51.5	51.0	54.8	48.0	51.0	50.0	48.8	47.8	41.2	45.3	46.7	52.2	51.1	52.1	35.4	48.9	40.8	34.3	34.4	30.2
	D	52.6	51.3	54.0	53.6	52.4	46.8	45.0	48.0	44.2	.....	.....	53.1	56.4	53.4	52.0	51.3	52.0	47.5	49.4	44.5	.....	.....
	E	40.0	48.1	47.4	47.9	47.7	45.8	45.4	43.2	43.2	36.6	.....	49.1	45.8	42.5	36.4	30.0	21.3	22.0	16.8	17.4	16.8	.....
8	A	51.2	57.4	59.8	61.0	63.2	60.0	57.6	57.8	54.7	52.8	49.3	38.8	40.1	38.4	45.5	45.0	37.2	40.1	33.3	21.4	17.5	9.2
	B	58.6	61.7	62.0	63.2	61.9	60.4	58.0	56.9	51.3	.....	.....	44.4	48.6	45.0	44.4	40.0	39.6	24.9	32.8	21.1	.....	.....
	C	54.0	61.4	61.3	62.1	59.9	59.6	48.9	57.6	55.0	49.2	.....	48.2	50.6	47.1	50.0	50.0	47.9	52.3	46.7	47.1	38.8	.....
	D	26.3	50.0	47.1	50.1	45.6	47.2	47.3	40.7	43.6	32.0	.....	18.6	48.2	38.9	41.2	28.2	23.4	18.4	15.2	16.1	15.6	.....
	E	42.2	46.3	51.4	45.7	48.3	51.4	48.9	46.2	43.9	37.8	.....	40.8	39.1	43.1	22.7	32.1	40.4	26.0	23.6	17.0	16.2	.....
9	A	50.3	58.9	61.6	57.6	57.4	56.7	54.5	52.5	48.1	36.8	.....	38.2	42.0	39.2	23.8	20.4	19.0	16.0	14.1	13.0	13.8	.....
	B	34.1	44.0	42.0	43.5	43.8	43.3	40.7	43.6	38.9	37.3	35.8	14.1	16.0	12.5	12.6	12.9	12.5	12.7	11.2	12.8	14.5	12.2
	C	30.9	58.5	61.2	60.6	56.7	59.2	55.4	53.4	50.8	49.4	42.9	46.2	48.8	48.4	46.9	44.6	40.9	40.0	33.9	22.6	26.5	17.0
	D	37.6	55.0	60.5	60.6	60.5	59.4	57.8	57.7	54.8	51.1	50.4	33.8	41.8	46.6	46.5	45.0	39.7	35.9	29.8	25.7	15.3	17.0
	E	48.4	49.6	50.3	48.2	42.0	43.3	41.0	37.8	.....	.....	.....	48.7	47.1	46.1	41.8	16.5	15.8	16.5	14.7	.....	.....	.....
10	A	32.5	44.0	47.0	44.6	42.2	42.6	42.2	37.5	25.8	.....	.....	15.6	17.9	14.2	13.9	15.8	20.2	13.9	14.2	14.6	.....	.....
	B	36.0	59.0	60.1	61.8	59.6	58.6	58.5	54.0	52.8	45.8	.....	13.9	28.9	31.5	24.4	21.3	22.1	17.1	15.4	18.6	17.0	.....
	C	42.6	49.5	49.7	51.8	50.4	49.8	48.6	45.5	42.7	38.3	.....	16.1	21.4	14.9	18.8	16.8	17.3	17.2	14.9	13.2	12.4	.....
	D	50.5	.....	53.2	52.8	50.7	48.7	45.7	41.6	.....	.....	.....	40.2	.....	40.5	41.7	32.5	23.7	21.5	17.3	.....	.....	.....
	E	38.2	48.4	44.8	42.0	42.0	41.0	41.5	39.8	35.0	.....	.....	17.1	15.8	13.3	12.5	12.7	11.6	12.4	12.3	12.9	.....	.....
11	A	55.7	56.0	63.8	62.8	59.9	59.4	56.7	50.4	49.8	37.6	.....	19.5	22.8	33.3	26.9	19.7	17.0	16.0	13.7	13.5	14.5	.....
	B	51.9	60.5	62.1	59.6	62.3	62.8	60.4	55.8	50.3	52.9	33.2	27.9	34.2	26.8	19.1	24.6	24.0	17.7	14.9	14.7	14.4	16.4
	C	46.1	57.0	54.7	55.4	56.0	56.0	54.4	.....	44.5	44.2	.....	24.4	30.4	29.7	18.5	20.9	13.8	16.2	.....	15.2	14.7	.....
	D	42.6	42.7	43.6	43.3	40.5	38.4	38.8	36.1	43.1	34.6	.....	15.0	13.4	12.8	13.2	12.5	12.5	13.0	13.2	12.9	12.6	.....
	E	56.4	63.2	63.2	62.4	61.4	60.9	56.2	54.4	49.2	40.4	.....	34.1	45.3	42.4	40.2	36.8	30.0	24.4	17.8	15.0	14.8	.....
12	C	52.7	59.8	61.5	57.6	56.7	57.0	55.9	52.2	52.1	44.3	.....	34.4	37.0	24.8	20.0	15.6	13.1	12.9	12.1	12.1	11.7	.....

The distribution in percentage of water (fig. 13, 14, 15) is much more uniform. Just at flowering time there is considerable variation, due, doubtless, to exceedingly rapid changes taking place at that time.

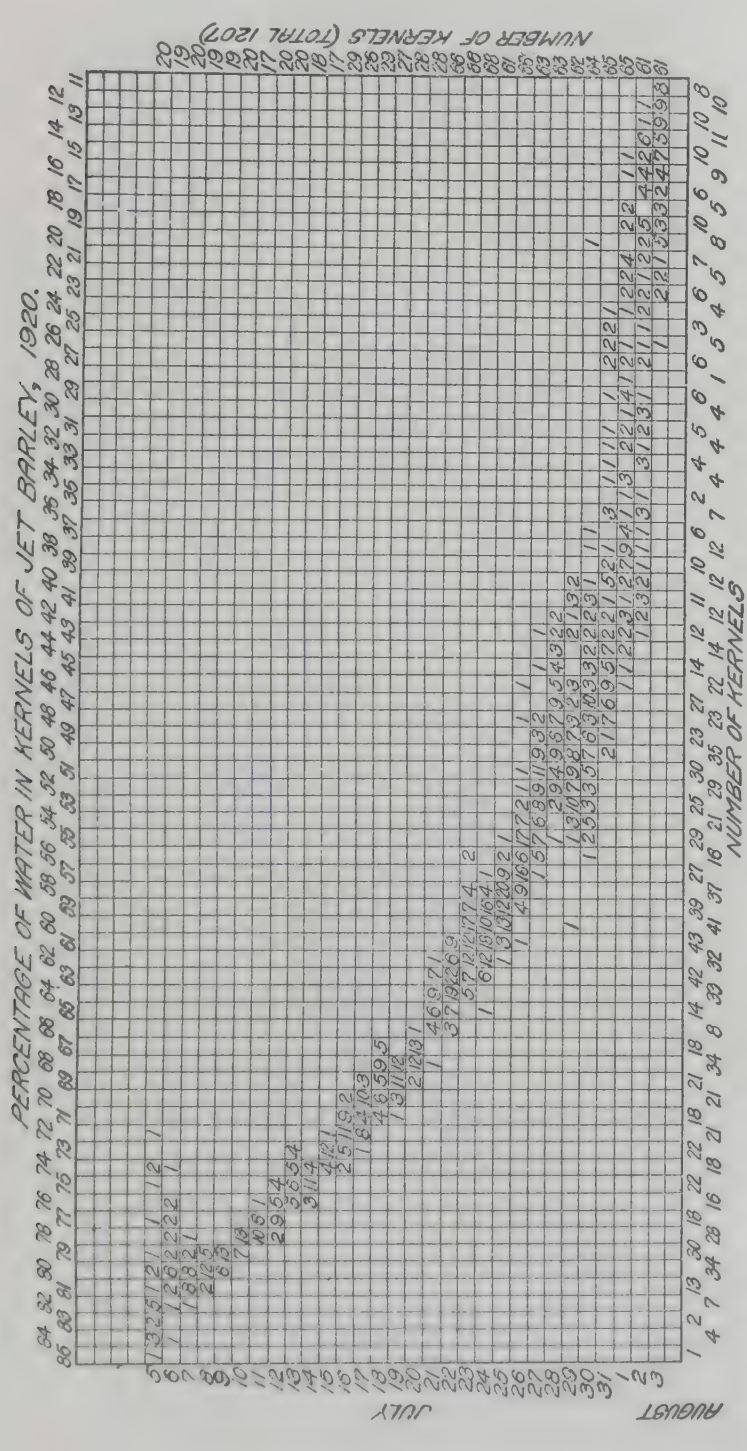

FIG. 13.—Distribution of kernels of Jet barley according to the percentage of water by days from flowering to maturity at Aberdeen, Idaho, in 1920.

Immediately following flowering the kernels show almost identical water content for any one day and almost identical daily decrease in water content for many days. The exact date of the beginning of spread in the distribution is difficult to determine. It is evident that it is not much before the seventeenth to twentieth day. By the twenty-third day

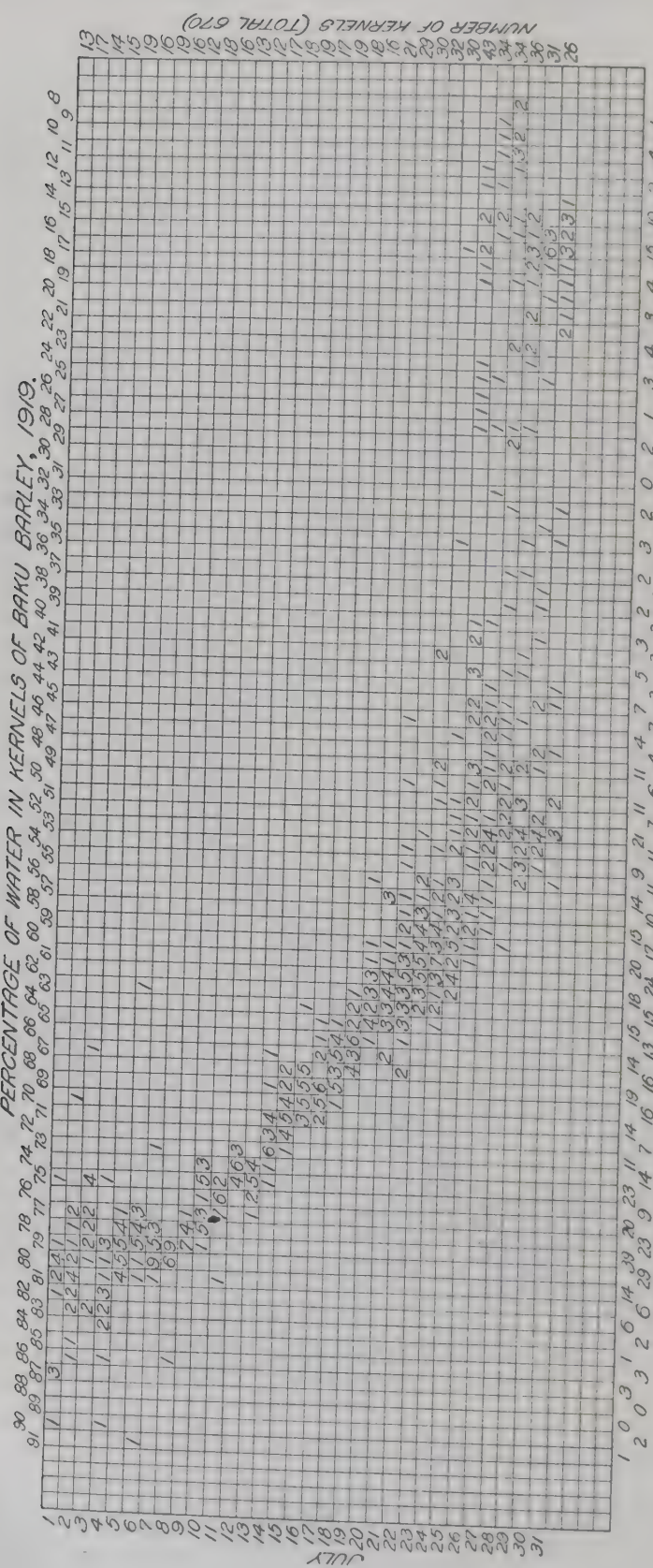

FIG. 14.—Distribution of kernels of Baku barley according to the percentage of water by days from flowering to maturity at Aberdeen, Idaho, in 1919.

ripe kernels are in evidence. The changes apparently take place at a higher water percentage in the Baku than in the Jet variety. In the Baku the kernels dry very rapidly after they have reached the moisture

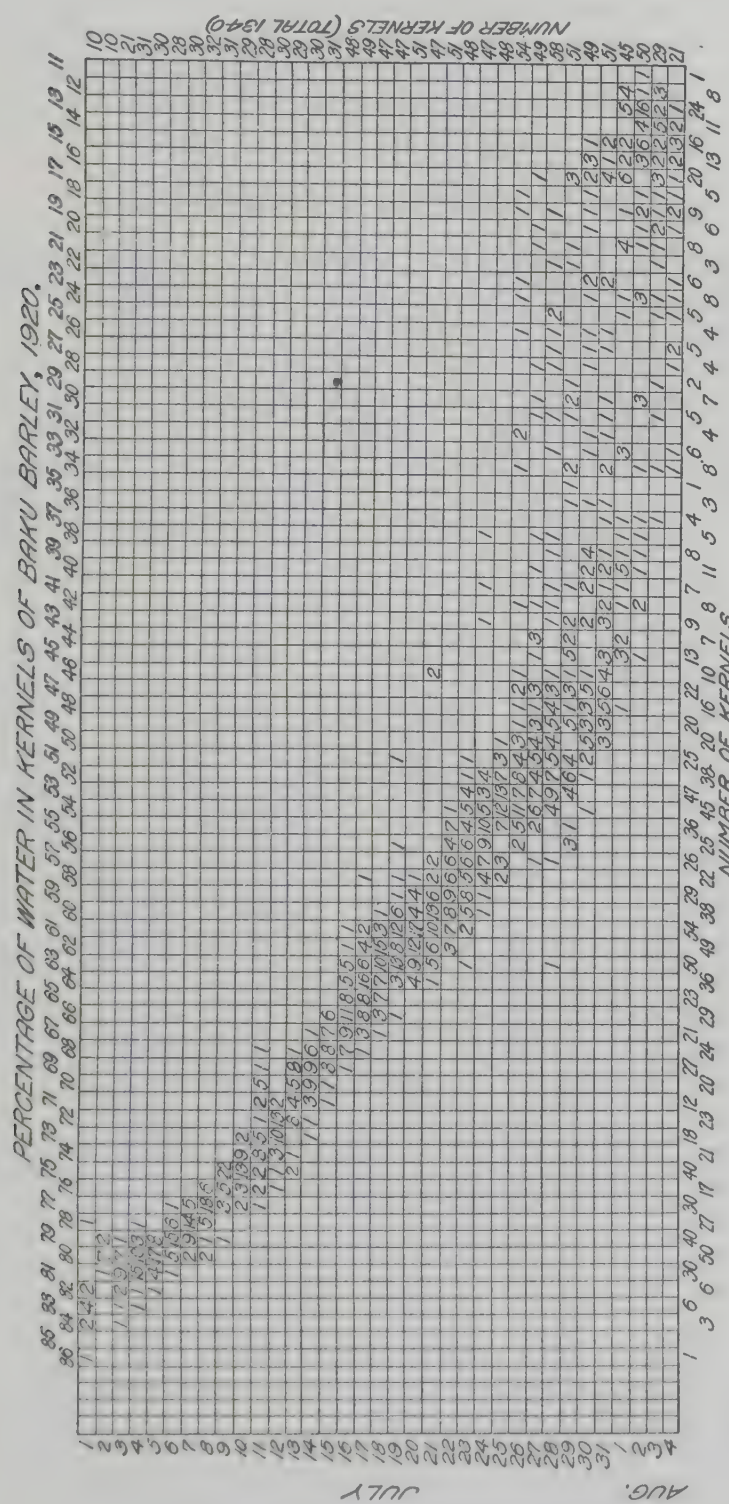

FIG. 15.—Distribution of kernels of Baku barley according to the percentage of water by days from flowering to maturity at Aberdeen, Idaho, in 1920.

content of 50 per cent. There are few kernels between 42 per cent and 20 per cent, showing that mechanical drying is taking place with great rapidity.

Thus far the data for the Jet and Baku varieties are quite comparable to those reported earlier. No conclusions can be drawn which were

not apparent in the previous studies As has been pointed out, the sudden break in the curve of percentage of water, which has held a steady trend from flowering, could come about only through the abrupt termination of growth. It is not evident from these or previous figures whether this abrupt termination involves the whole kernel at once or whether there has been a final abrupt termination of activity in a limited area preceded by a gradual abandonment of tissue to the point where the active tissue remaining affords an insufficient volume in which to take care of the starch produced daily by the plant.

COLOR AS AN INDEX OF MATURITY

Although the figures, taken by themselves, do not afford sufficient basis for the analysis of the final phases of maturation, the color formed in the Jet variety offers a suggestive clue. The melanin-like pigment is apparently deposited only when activity has ceased. In the normal kernel the color appears just before final maturity. The earliest traces are found about the twentieth or twenty-first day after flowering. If a kernel is removed from the spike even six days after flowering, the black color will appear on drying. It seems reasonable to suppose then that when this color appears on the kernel, the section of the kernel immediately beneath it has ceased to function. That this assumption is plausible is evidenced by two related observations. The black color appears first on the dorsal surface just below the tip. The tip of the kernel up to this time consists of active tissue remaining from the ovary walls. As soon as the black color appears, this tissue withers, showing that the vascular system here has ceased to function. This peculiarity has been noted in other years, when it had been found possible to measure the true length of the kernel only after the withering of the tip. Previous to that time the measurement necessarily had to include tissues which had no real connection with the growth inside the nucellus. It was also in this region that cells showing early abandonment as active repositories for starch are most numerous, indicating that this region was the first to show lack of free movement of cell solutes.

The progress of color formation and loss of water, as evidenced by the denting and collapse of the kernels, is very definite. Traces of color appeared when the moisture content was about 62 per cent. It was present on most kernels by the time they had reached 60 per cent. The spot was very definitely marked when they had reached 58 per cent, and the area had spread well toward the end of the kernel when the water content had reached 56 per cent. Denting appeared at about 55 per cent and was common at 51 to 52 per cent. Color appeared on the ventral surface of the grain at 53 per cent and was on the ventral surface of most kernels by the time they had reached 48 per cent. The sides as well as the dorsal surface had begun to dent at about 50 per cent and were commonly dented at 46 per cent. The ventral surface was black with the exception of the furrow on some kernels at 46 per cent and commonly so at 43 per cent. Even the furrow was black in some instances at 42 per cent. Few furrows with traces of green were found under 40 per cent. Fine wrinkles appeared all over the kernel at percentages about 30 and on all kernels containing below 24 per cent of water.

When these color observations are considered with the data previously presented, they assist materially in the interpretation. The black area on the dorsal surface became marked about the twenty-second day after

flowering—that is, the average kernel was affected by this date. On the curve of dry weight (fig. 10), it can be seen that the rate of increase fell off markedly on this date and never again reached the rate held consistently for a long period previously. The water content began to drop very rapidly at this time, as did also the wet weight. It seems without question that the abandonment of tissue began actively when the water content of the average kernel had reached 58 per cent. On some kernels this change evidently was initiated at 62 per cent. Dry matter was added, however, to other parts of the kernel for several days. Growth was essentially complete when the color had covered the ventral surface. This occurred when the kernel had reached 46 per cent of moisture and was common on all kernels when they had reached 42 per cent. This 42 per cent coincides with the average date reported in various previous papers, and in this instance it is coincident with the average appearance of color. It would seem that, since 42 per cent represented the average water content when the spike was mature, this percentage would also represent the point at which the individual kernel should cease activity. That this is not true is due to the variation of spread before and after reaching maturity. Once a kernel has ceased active metabolism, the loss of water is very rapid and the spike average is lowered by wide range of water content in those kernels already mature.

While not all kernels showed the appearance of black at the same time, the earliest kernels exhibited the color when a water content of about 62 per cent was reached. From the small spot first occurring the color gradually spread over the dorsal surface of the kernel. The base of the kernel was last affected on both the dorsal and ventral surfaces. On the ventral surface, color first appeared on the flanks of the grain from the center of the kernel toward the tip. The green in the pericarp disappeared last from the furrow of the kernel and naturally this is the last active tissue. Shortly after the black color appears on the dorsal surface, the kernel begins to dent—that is, the loss of water in this region is sufficiently rapid to cause the tissue to collapse. The loss of volume at this time is apparent in the measurements of the grain. The kernels of all varieties of barley contract as they lose water in maturing. In the Jet variety the kernel collapses in a depression on the dorsal surface which reaches from the tip to the embryo. This is not common in barley varieties and doubtless comes about through the fact that in the Jet the tissues external to the nucellus are abnormally thickened in this region. Since such external tissues are not utilized for storage, the loss of water must be accompanied by an unusual reduction in volume. The final stage of ripening, or rather mechanical drying, is accompanied by a fine wrinkling of the entire surface of the kernel, as is common in other varieties.

Assuming that the kernels of all varieties ripen in the same way as those of the Jet, the data here presented add this much to our previous information in maturation. The process of ripening is abrupt, as has been previously apparent, but this final stoppage of activity is the culmination of a maturation which has already checked activity in some parts of the kernel. As may be seen in Figure 4, the drop in the average percentage of water is not accelerated by the abandonment of the limited portion of the kernels first affected. It is only when the black spot becomes common, indicating an average condition of abandonment, that the rate of loss of water is accelerated and the rate of deposit of dry matter checked. From this time there is a slight increase in average

dry matter. This increase doubtless is exaggerated in the averages due to the fact that all kernels are not equally advanced.

The date of maturation of individual kernels is partially dependent upon minor features of location. Supposing that a kernel can not be rejuvenated after the percentage of water has fallen below 44 per cent, the time of reaching this percentage may be postponed by protection from the sun and air. The loss of water from the kernel is active on hot days. There is no loss at night during growth. Very hot, dry, sunny days may lower the water of exposed kernels near maturity below the point of recovery. Those kernels well covered by the awns of other kernels ripen slowly. The kernels on the under side of the spike of nodding varieties ripen after those on the upper side. In varieties like the White Smyrna, where several kernels are inclosed in the leaf sheath, the kernels so inclosed ripen much later than the exposed kernels. Prolonged cool weather at ripening time must have a similar effect, and high yields of plump grain usually result from such ripening periods. These observations fit in with the appearance of the color in the Jet variety. While the amount of light reaching the kernel affects the intensity of the pigment and occasionally the time of its appearance, in general it affords an accurate index of the stage of maturity.

SUMMARY

In previous studies it has been shown that the average water content of the ovaries at flowering time is about 80 per cent and that the percentage of water in the growing kernel decreases uniformly day by day until the average for all the kernels of a spike is about 42, when all deposit of dry matter is interrupted and the kernels dry with great rapidity.

This 42 per cent does not represent the exact point where translocation of material becomes impossible but is lower than this point, as this is the average of all kernels on the spike and must include some with slightly more water which are still functioning and some with much less which ceased to function a day or two previously.

Endosperm cells mature abruptly; the proteid content probably reaches a density beyond which it can not function. The Jet is a naked variety in which a black pigment is formed in the pericarp whenever this tissue ceases to be active. The appearance of color indicates that the first region to mature is on the dorsal surface near the tip. The region of the embryo on the dorsal surface is still later, and the cells adjacent to the furrow on the ventral surface are the last to mature. The first cells on the dorsal surface are affected when the moisture content of the kernel has reached 60 to 62 per cent. The kernels are fully mature when the water has fallen to 46 per cent and have carried on only a limited amount of translocation for some time.

The date of final maturation can be postponed and the size of the kernel increased where kernels are protected by leaf sheaths or other shade and by cool weather at ripening.

BACTERIAL LEAFSPOT OF GERANIUM IN THE EASTERN UNITED STATES[1]

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The florist's season for growing cuttings of geraniums (*Pelargonium* spp.) for garden and window-box planting is the season when this bacterial disease may be looked for, especially in crowded, ill-ventilated houses. Nearly every spring since 1912 and at any time during March, April, and May, according to the locality where the plants are grown, we have received diseased specimens. Very rarely has a diseased plant grown in the open been sent to us. Our first acquaintance with it, however, was at an earlier date when it occurred on the grounds of the United States Department of Agriculture in Washington (Pl. 1, A). In New Jersey and Maryland the disease has been especially prevalent and of a destructive nature, whole houses of rooted plants in some instances becoming unsuitable for market. It is a spot disease which makes the leaves unsightly and undermines the health and development of the cuttings. The year 1915 was evidently favorable for the disease, for it occurred very generally in the eastern part of the United States.

The spots are definitely outlined, usually irregular but occasionally circular in shape, and of a brown color. The spotting may begin either at the margin of the leaves or on the blade and may occur on either old or young leaves, although the older leaves or those nearest the soil are usually the worst affected. The youngest infected areas are watery looking, then become a reddish brown, later the tissue dries and becomes dark brown. When the disease is advanced the portion of the leaf between the spots turns dark and becomes dry and wrinkled. The spots, however, still show plainly in the dead area. The bacteria, which are motile, occur in great numbers in the spots. They are easily isolated from the young spots, as the epidermis can be sterilized with mercuric chlorid (1 to 1,000) for three to five minutes without much penetration.

Isolations were made from material received from Maryland (Pl. 1, B), and water suspensions of subcultures were sprayed on young geranium plants growing in the greenhouse. The plants were kept in a moist infection cage for two days. In another cage, which was also kept moist, were other geranium plants of the same age which were sprayed with sterile water. Typical spots appeared on the inoculated (sprayed) leaves in 9 days, and in 17 days the plants were badly spotted (Pl. 3, B). The controls did not show any spotting. Bacteria were abundant in the spots. The organism was reisolated and other geranium plants were infected with it by spraying. In four days spots began to show on the leaves of these sprayed plants. The temperature was higher at this time, being 80° F. during most of the daytime.

[1] Accepted for publication May 10, 1922.

Likewise, isolations were made from diseased material from New Jersey (Pl. 2). The same organism was obtained and typical spot produced in 17 days from the time of spraying the leaves with a wate suspension of an agar subculture (Pl. 3, A). The organism was reiso lated also from this New Jersey strain. The inoculations with the reiso lated New Jersey organism were made in two different greenhouses, one in which the day temperature was 70° to 75° F. and another in whicl it was 55° to 60° F. The plants were sprayed and kept in infection cages for two days. In the warmer house infection occurred early and was well marked in 16 days, while there was but a trace of infection on the inoculated leaves in the cooler house. The disease did not progress in the latter case but continued in the former.

A bacterial disease of geranium plants was reported from Massachusetts [2] in 1898. The observers first found it on several different varieties during a season of rainy weather about the latter part of July on plants grown out of doors. The leaves were spotted and bacteria were found in the spots.

Another report of the disease from Massachusetts [3] stated that attempts had been made to isolate the organism but without success.

Still another report [4] said the disease had been noticed in that State every year for nine years. It was abundant and generally distributed, and gardeners had become concerned about it. The spotting was not serious in greenhouses, however, and it was thought to have been brought in from out-of-door stock.

Dr. Erwin F. Smith,[5] in volume 1 of his "Bacteria in Relation to Plant Diseases," mentioned the disease as one produced by stomatal infection and in volume 2 called it a disease of rainy seasons. In volume 2 he also stated that the organism was isolated in his laboratory and the disease reproduced on geranium leaves by John R. Johnston, inoculating from pure cultures. No further work on this disease was done by Mr. Johnston or by Dr. Smith beyond recognizing it as a yellow organism with a polar flagellum. He states to me that none of the earlier isolations from the Washington material ever greened the medium.

A bacterial disease of the leaves of Erodium and Pelargonium in Texas was described in Phytopathology [6] for August, 1914. The general appearance of the spots on the leaves in the illustrations of the article led us at first to think that the Texas disease described was the same as the one our laboratory had been dealing with, although Lewis's figures 2 and 3 indicate a much more active parasite. When comparisons of cultural tests were made and what seemed to be important differences were noted, it was decided that the two organisms must be different and that these different features would be accentuated if worked out in more detail. That has been done, and careful observation and repeated tests have established the writer's belief that the organisms are not the same, for in cultural tests important differences persist. Perhaps Mr. Lewis's work was done with the Erodium strain and the host may be responsible for these differences. He does not state in his paper which isolation he

[2] STONE, George E., and SMITH, Ralph E. A DISEASE OF THE CULTIVATED GERANIUM. *In* Mass. Agr. Exp. Sta. 10th Ann. Rpt., 1897, p. 67, 1 pl. 1898.

[3] ——— ——— A GERANIUM DISEASE. *In* Mass. Agr. Exp. Sta. 12th Ann. Rpt., 1899, p. 57–58. 1900.

[4] STONE, G. E., and MONAHAN, N. F. BACTERIOSIS OF GERANIUMS. *In* Mass. Exp. Sta. 19th Ann. Rpt., p. 164. 1907.

[5] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. V. 1, p. 92, 1905; v. 2, p. 39, 62, 1911. Washington, D. C. Carnegie Inst. Publ. 27.

[6] LEWIS, I. M. A BACTERIAL DISEASE OF ERODIUM AND PELARGONIUM. *In* Phytopathology, v. 4, no. 4, p. 221–232, pl. 10. 1914.

used for his cultural tests. He states that he isolated an organism from both Erodium and Pelargonium and that he was convinced that the organism from the wild Erodium and the cultivated species of geranium (Pelargonium) were one and the same. He found that both strains cross-inoculated readily but does not mention making any comparative cultural tests of the two strains. In all probability he used one strain only for his cultural tests.

The comparisons in this paper were started with the idea of finding enough agreement in the cultural tests to establish the identity of our organism as *Bacterium erodii*, and with that in view we cast about for explanations to account for the differences. One striking difference between the Texas and Maryland organisms is in the production by the Texas organism of a green fluorescence in beef agar, beef bouillon, sterile milk, and various other media. Very careful observations were made, but no trace of green fluorescence could be detected at any time in the tests with the Maryland or New Jersey isolations. The continued comparisons in this paper were made with the New Jersey isolation. Morphologically the Texas and New Jersey organisms are much alike and in some of the cultural tests are identical. Although in two to five days we have never obtained leaf-spots such as Lewis figures the two organisms produce somewhat the same type of disease on geranium leaves apparently under the same conditions, yet if they are the same organism why does one produce green pigment and the other not?

Thinking the green color might have formed through some particular property of the medium, two lots of peptone-beef bouillon (beef infusion) were made in which Witte's and Difco peptone were used as well as two lots of beef extract media with the two kinds of peptone. Our organism acted the same in all in respect to color—there was not a trace of greening. Then Mr. Lewis's platings from diseased material were considered. At the start an organism appeared on his plates which greened the agar. Subcultures from these colonies picked from the plate produced the disease. Could this pigment formation be the individuality of a strain? If so, it is a striking variation and a feature that must be reckoned with when comparing strains and varieties in proving up a new organism.

The New Jersey strain of the geranium leafspot disease was used for the tests described hereafter in this paper. To facilitate comparison with *Bacterium erodii* the same order is followed here as in the article by Lewis describing that organism.[7] No work was done with *Bacterium erodii* itself, as we had no culture of that organism nor were we able to obtain Texas material for our own isolations. The comparisons, therefore, were all made with the rather full tests published by Mr. Lewis in the article just cited.

CULTURAL CHARACTERISTICS OF THE ORGANISM FROM THE DISTRICT OF COLUMBIA, MARYLAND, AND NEW JERSEY

AGAR PLATES.—On beef infusion peptone agar +16.5 plating from a 2-day-old bouillon culture, colonies do not appear until the third day. In reflected light they are cream color, shining, round with a smooth surface. In transmitted light under a hand lens they are a cream color in the center and bluish outside of center. There are delicate reticulate

[7] LEWIS, I. M. OP. CIT.

markings in the interior; there is no zoning, but a marginal ring occurs on some colonies after they are up several days. Colonies are 2 to 4 mm in diameter. The agar does not change color. (Unlike the Lewis organism.)

AGAR STROKE.—In 2 days there is a translucent wet shining rather thin growth on +13 peptone-beef infusion agar. In 3 days the growth is cream colored with undulating surface. There is no green color in the agar or condensation water. The surface is finely pitted at 4 days; crystals form in from 2 to 10 days. There is no viscidity until cultures are 1 month old or older, but even then there is no green color. (The Lewis organism produces a green pigment.)

GLUCOSE AGAR STAB.—This is a favorable medium. Growth is fairly rapid and abundant on the surface of the stab; scant along the line of puncture. The color of 4-day-old cultures is Ridgway's Naples yellow,[8] and this is a deeper color than the growth on plain beef agar. (Same as the Lewis organism.)

LACTOSE AGAR STAB.—In this medium there is growth with abundant crystals. No green color. (The Lewis organism produces a green color.)

STEAMED POTATO CYLINDERS.—A thin yellow growth occurs on the potato in 24 hours. In 2 days it is a Naples yellow; in 3 days the potato begins to darken a little, but there is no green fluorescence. The potato cylinders are still firm after a month. The starch in the cultures gives a purple reaction when tested with iodin in potassium iodid. (Lewis's organism produces a green fluorescent pigment after 48 hours.)

STEAMED COCONUT.—A thin faintly yellow growth occurs in 2 days on pieces of coconut steamed in tubes. Growth takes place in the water also but is not a yellow color. In 2 weeks the growth on the surface of the coconut is still thin and not viscid. (The Lewis organism produces a viscid growth on this medium.)

LITMUS MILK.—There is a trace of clearing (whey) in 3 days but no color change. In 6 days there is a faint bluing in bands and a clearing at the surface for nearly 1 cm., but no coagulation. In from 10 to 15 days the bands of color are faint and clearing has taken place in from one-half to the entire tube. Then it is a reddish blue color, dark hyssop violet, according to Ridgway. (With Lewis's organism the liquid is all a clear yellowish color in 8 days.)

STERILE MILK.—No clearing occurs before 5 days even at the optimum temperature of 26° to 28° C. At that time there is a ring of whey, cream colored, 3 to 5 mm. deep. Coagulation comes soon after the clearing appears; the curd is soft. There is no color change. Tested at 17 days the hydrogen-ion concentration expressed in P_H value is 6.4 (Brom cresol purple). In 30 days over half the curd has been digested. (The Lewis organism does not coagulate sterile milk; it produces green fluorescence with age.)

DUNHAM'S SOLUTION.—There is heavy clouding in this medium in 48 hours, but not any marked difference from peptone bouillon. (Like the Lewis organism.)

PEPTONE BOUILLON.—The organism clouds peptone beef infusion bouillon +10 to +15 in 24 hours at a temperature of 23° to 27° C., when transfers are made from a young fluid culture. At 18° to 20° it is not clouded until 48 hours. Clouding is heavy in 2 days at 27°. Pseudo-

[8] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

zooglea are present. In 2 to 4 days an incomplete pellicle is formed and the medium is clear below the pellicle. Cultures are not viscid until 1 month old or older. When the tube is agitated the pellicle drops to the bottom in flocks. No green color is produced in either young or old cultures. Some bouillon was made containing Witte's peptone, and the organism grown in it was compared with that containing Difco peptone to see whether the green color would appear. It did not, nor when extract of beef was used with the two kinds of peptone instead of the beef stock. There was no trace of a green color at any time. (The Lewis organism greens the bouillon.)

DUNHAM'S SOLUTION WITH METHYLENE BLUE.—The organism grows well in Dunham's solution colored with methylene blue. The color changes to a faint blue in four days. This test was repeated with the same result. The color reduced from the bottom of the tube upward. In 22 days the methylene blue is reduced to a mere trace. In 1 month after inoculating the color begins to return, but is a green instead of blue—light hellebore green, according to Ridgway.[9] (The Lewis organism does not change the blue color; observations were made over a period of 4 weeks.)

BOUILLON WITH SODIUM CHLORID.—Growth takes place in neutral beef bouillon to which 2.5 per cent sodium chlorid is added. Tests were made with 3.5 and 4 per cent sodium chlorid, but no growth took place in either of the latter media. (Like the Lewis organism.)

BOUILLON OVER CHLOROFORM.—No growth occurs in 10 cc. of peptone-beef bouillon over 5 cc. of chloroform. Three different tests were made, one in which the quantity of chloroform was reduced to 3 cc. No growth occurred. The same bouillon inoculated without chloroform showed abundant growth in 48 hours. (The Lewis organism grows in this medium.)

DECOCTION OF GERANIUM LEAVES.—Fifty gm. of fresh geranium leaves were boiled in a liter of water, then filtered and autoclaved. It tested +6, Fuller's scale; P_H 4.1. The organism was slow in appearing and grew but feebly in this medium. (Not unlike the Lewis organism.)

GERANIUM AGAR.—Some of the decoction described above was made up into agar by adding 2 per cent agar. The medium titrated +6 Fuller's scale; P_H 4.1. A mere trace of growth occurred in the agar made from the decoction. (Not unlike the Lewis organism.)

NITRATE BOUILLON.—There is slight growth in 2 days in beef bouillon containing 1 per cent potassium nitrate; good growth in four days. A partial pellicle is formed rather heavy with crystals. This pellicle falls apart in handling the tube. (The Lewis organism makes a persistent pellicle.)

FERMI'S SOLUTION.—In 2 days there is a faint clouding at temperatures of 25° to 28° C. The clouding is still faint in 2 weeks. In older cultures an incomplete pellicle is formed. There is no green fluorescence with age. A culture 3 months old is Ridgway's old gold color. (The Lewis organism produces green fluorescence.)

COHN'S SOLUTION.—There is no growth in Cohn's solution. (The Lewis organism grows in Cohn's solution without greening.)

USCHINSKY'S SOLUTION.—At temperatures 25° to 28° C., which are favorable temperatures for this organism, there is only a mere trace of growth in 2 days, and no heavier at 7 days. In 2 weeks clouding is better,

[9] RIDGWAY, Robert, op. cit.

and later an incomplete pellicle forms. There is no change in the color of the medium when cultures are 3 months old. (The Lewis organism grows promptly in this medium without greening.)

GELATIN STAB.—With +10 beef-infusion gelatin, stab and plates, the liquefaction occurs slowly. Plates thickly sown show slight liquefaction in 7 days, and most of the plate liquefies in 12 days. Temperature 18.5° to 20° C. The liquefaction starts in the stab cultures in 6 days and continues slowly across the surface. In 1 month they are about one-fourth liquefied. In 2½ months the stabs are slightly over half liquefied. In 3½ months one stab was entirely liquefied and the others three-fourths. In 4½ months all cultures are entirely liquefied. (The Lewis organism liquefies a stab culture in 4 weeks.)

LACTOSE LITMUS AGAR.—Growth takes place readily. There is no color change in 5 days. In 7 days the bacteria have taken up color, and a mass on a platinum loop looks green. The slant has become blue, but the agar at the bottom of the tube has not changed color. In 16 days the color of slant is still blue; that part of the medium in the bottom of the tube is unchanged. (The Lewis organism reddens the medium.)

STEAMED CARROT CYLINDERS.—Growth does not take place quickly on carrot cylinders, but in 12 days the surface of each is covered with a creamy growth, smooth, wet shining, not viscid. There is no browning of the medium in 25 days. (The Lewis organism browns the medium.)

STEAMED WHITE TURNIP.—Growth is slow in starting on this medium and is always thin. It is creamy in color, wet shining, not viscid. The medium is neither softened nor browned in 25 days. (Mr. Lewis says this medium is favorable for growth and that it becomes soft and brown.)

INDOL.—There is a slight production of indol in Dunham's solution cultures 10 days old. It is still slight when the cultures are 18 days old. The tests were made with sulphuric acid and sodium nitrite. (Like the Lewis organism.)

HYDROGEN SULPHID.—Hydrogen sulphid is produced. The organism was grown on potato cylinders, beef agar, lactose agar, and in beef bouillon. The test was made by suspending lead acetate paper in the culture tubes. The paper became well blackened in every case. (The Lewis organism does not produce hydrogen sulphid in any of these media, not even after prolonged exposure.)

AMMONIA PRODUCTION.—The organism produces ammonia. Cultures of beef bouillon and peptone water both 10 days and 3 weeks old were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. The cultures were then heated in a water bath. A red-brown color appeared on the filter paper immediately. (Same as Lewis's organism.)

NITRATE REDUCTION.—There is no reduction of nitrates to nitrites. Tests were made with nitrate bouillon in which the organism grew very well. Ten-day and 28-day cultures were tested. *Bacillus coli* grown in the same medium and tested by the same method (starch-iodin sulphuric acid test) gave a positive test. (Same as Lewis's organism.)

REDUCTION OF LITMUS.—Litmus is reduced in 10 to 15 days in sterile milk. (Not appreciably different from Lewis's organism.)

METHYLENE BLUE.—Reduction of methylene blue takes place in 7 to 11 days, according to temperature. Tests for the reduction were made in milk colored to a robin's egg blue. The reduction begins from the bottom of the tube and goes upward. In 3 days it is white at the bottom of the tube for one-eighth of the liquid. The rest of the liquid is a slightly lighter blue than the control. No coagulation occurs. In 11 days there is entire reduction in some tubes. When entirely reduced the milk coagulates. Those tubes with a slight blue at the surface are not coagulated. In 15 days the blue in all tubes is wholly reduced. Temperature 20° to 22° C. When tubes of methylene blue milk are inoculated and placed at 27° to 28° reduction occurs throughout in 6 days. (With the Lewis organism the tests for the reduction of methylene blue were negative.)

THERMAL RELATIONS

THERMAL DEATH POINT.—The thermal death point lies between 51° and 51.5° C. Transfers were made from well-clouded 24-hour-old cultures and tested many times. The beef bouillon titrating +13 to +17 on Fuller's scale (P_H 6.8 to 7.0) was held in thin-walled test tubes and after transfers were made was kept and heated for 10 minutes at constant temperature in a water bath. Growth occurred at all temperatures tried (48°, 48.5°, 49°, 49.5°, 50°, 50.5°, 51°) except 51.5°. Once in six tubes there was no growth at 50°, but in another six tubes all grew at 50.5°. In one out of two tests growth took place at 51°. None at 51.5°. (The thermal death point of the Lewis organism is 48.5°.)

OPTIMUM TEMPERATURE.—The optimum temperature is around 27° C. Tests were made with temperatures from 10° to 40°. The organism clouds +15 bouillon in 24 hours from 23° to 28°, but in 48 hours the growth is heavier at 26° to 28°. (Agrees with the Lewis organism.)

MINIMUM TEMPERATURE.—Growth occurs at 1° C. In one test with the organism grown in +15 beef bouillon there was a trace of clouding in 16 days. The temperature ranged from −1° to 1.75°. In a second test in which the thermostat ranged from 0.6° to 1.25°—never over 1.25° and usually under 1°—growth was visible in 49 days. It was never more than a faint clouding. (The Lewis organism grows in both beef agar and beef bouillon at 0°.)

MAXIMUM TEMPERATURE.—Growth occurs at 35° C, but it is very feeble. There is none at all at 36° to 37°. (Very little difference from the Lewis organism.)

TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. Agar and bouillon transfers were placed in a specially devised jar from which the oxygen was removed as follows: A solution of 35 gm. of potassium hydroxid dissolved in 350 cc. of water was poured over 40 gm. of pyrogallic acid. The bottle for this solution had previously been adjusted in a jar where the transfers were. The mixture was poured in the bottle and its top left uncovered; but the jar was covered, then another cover inserted in a bed of mercury was placed over the whole. A tube of methylene blue milk and litmus milk had been placed in the jar with the cultures as controls on the presence of oxygen.

No growth occurred in any of the cultures in the jar. The bouillon control in the room showed good growth. The methylene blue had faded considerably in 11 days. The litmus did not fade until 15 days after setting up the experiment. A careful watch was kept for over 3 weeks, but no growth took place in the cultures until they were removed from the jar. (Like the Lewis organism.)

RELATION TO MOISTURE

This test was followed out according to Mr. Lewis's rod method. Glass rods were held in place in test tubes by passing them through the cotton plugs, after which the tubes were sterilized. Next the rods were dipped to a uniform depth in a 6-day-old bouillon culture, returned to the tubes, and left to dry at room temperature which varied from 18° to 23° C. Care was taken that the rod did not rest against the side of the tube and prevent a uniform drying. At intervals of 24 hours several of these rods were transferred each to a tube of beef bouillon. Growth occurred in one test after the cultures on rods had been dried 7 days. In a second test made exactly the same way, growth did not occur when dried 7 days but did occur at 6 days. Evidently 6 days is about the limit of drying for a 6-day-old culture of this organism. (Like Lewis's organism.)

GROWTH IN FERMENTATION TUBES

GAS FORMATION.—The organism is aerobic so far as tested and does not form gas. It was tested in fermentation tubes in the presence of each of the following carbon compounds: saccharose, dextrose, lactose, maltose, and glycerin; 1 per cent of these being added to a 2 per cent water solution of Witte's peptone. No gas formed in any of the tubes. Growth occurred in the open arm of each tube but none in the closed arm.

The cultures were tested for acidity after they had grown 21 days. Five tests were made—two in January, in which Witte's peptone was used in one and Difco peptone was used in the other. Another test containing Witte's peptone was made in June. The fourth and fifth tests were made in July with Witte's and Difco peptone, as shown by Table I. Tests 1, 2, and 3 are with Witte's peptone, 4 and 5 with Difco. The results are as indicated in Table I, and it is interesting to note how the same organism varies. With Difco peptone, the acidity of the medium was considerably reduced by the growing organism.

Titrations with phenolphthalein were made before inoculating and again after the organism had grown 21 days. The first test after 21 days showed there was little change in acidity in the cultures. The saccharose, dextrose, and glycerin cultures were slightly more acid than the controls; the maltose and lactose cultures were less acid. In the second test after 21 days all cultures were more acid than the controls. In the third test after 21 days there was no change in acidity with dextrose, lactose, and glycerin; there was increase with saccharose and decrease with maltose. The fourth and fifth tests after 21 days were made with Difco peptone. Titrations showed there was decidely less acidity in the cultures than in the uninoculated media.

To determine further the changes in acidity, some of each of the same medium was tested with the indicators brom thymol blue, brom cresol purple, phenol red or cresol red, before inoculating. This was done at the same time they were tested on Fuller's scale. In all five tests the

P_H values were found to vary from P_H 7.0 to 6.6—that is, from neutrality slightly to the acid side. After inoculating, vigorous growth occurred in all cultures. At the end of 21 days tests were again made with the indicators. There was no increase in the hydrogen-ion concentration. As indicated by the records (P_H 7.2 to 8.6) there was decrease in the hydrogen-ion concentration. In all five tests there was a uniformity of these changes in acidity, as shown by the indicators named above.

The titrations with phenolphthalein of cultures grown in carbohydrate media containing Witte's peptone were somewhat different from those made by Mr. Lewis with his organism, though not so strikingly so as with the Difco peptone. The second test was the only one that showed an increase in acidity throughout.

At the time of Mr. Lewis's experiments (his work was published in 1914) Witte's peptone was in general use in laboratory media.

TABLE I.—*Acidity of cultures of the writer's geranium leafspot organism*

Medium.	P_H.					Fuller's scale.				
	Witte's peptone.			Difco peptone.		Witte's peptone.			Difco peptone.	
	First test.	Second test.	Third test.	Fourth test.	Fifth test.	First test.	Second test.	Third test.	Fourth test.	Fifth test.
Saccharose:										
Uninoculated	7.0	7.0	7.0	7.0	6.8	+8.5	+9	+10	+10	+13
Inoculated 21 days	7.6	7.6	7.2	8.2	7.2	+12	+17	+15	0	+11
Dextrose:										
Uninoculated	6.8	6.7	6.8	6.8	6.6	+8.0	+10	+10	+11	+12
Inoculated 21 days	7.6	7.8	7.4	7.4	7.4	+16.5	+21	+10	+6	+8
Maltose:										
Uninoculated	6.9	6.8	6.8	7.0	6.6	+8.5	+9	+9	+11	+12
Inoculated 21 days	8.2	8.0	7.4	8.4	7.4	+4	+16	+7	−5	+8
Lactose:										
Uninoculated	6.8	6.8	6.8	7.0	6.6	+9.5	+9	+9	+10	+12
Inoculated 21 days	8.2	7.6	7.4	8.6	8.0	+8	+18	+9	−5	+8
Glycerine:										
Uninoculated	6.9	7.0	7.0	7.0	6.8	+8.0	+8	+9	+11	+14
Inoculated 21 days	8.1	7.6	7.4	7.6	7.4	+10.5	+15	+9	+3	+9
Peptone water:										
Uninoculated	7.0	No test.	7.0	7.0	6.8	+9.0	No test.	+9	+9	+12
Inoculated 21 days	8.1	...do...	7.4	8.4	7.4	+8	...do...	+7	−5	+8

SENSITIVENESS TO ACIDS AND ALKALIES

In uncorrected beef juice (titrating +23) part made alkaline with sodium hydroxid and part acidulated with hydrochloric acid the titration range of the organism is −6 to +28 (P_H 8.7 to 5.7).

Table I shows there is a reduction in the H-ion concentration after the organism has grown on the media 21 days when both Witte's and Difco peptone are used. A reduction of acid is indicated on Fuller's scale when Difco peptone is used, while with the Witte's peptone there is no uniformity of reduction or increase of acidity (indicated on Fuller's scale) though the table shows there are more cases of increase than decrease.

The Lewis organism increased in acidity from 4 to 8 points on Fuller's scale as Table II shows.

TABLE II.—*Acidity of Lewis's geranium leafspot organism*

Medium.		Fuller's scale.	Increase in acidity.
Saccharose	Uninoculated	+10	8
	Inoculated 21 days	+18	
Dextrose	Uninoculated	+10	7
	Inoculated 21 days	+17	
Maltose	Uninoculated	+8	4
	Inoculated 21 days	+12	
Lactose	Uninoculated	+8	8
	Inoculated 21 days	+16	
Glycerine	Uninoculated	+10	6
	Inoculated 21 days	+16	

In neutral beef bouillon acidulated to +22 with hydrochloric acid there is no growth. This test was made because it was not stated in Mr. Lewis's article what his foundation beef bouillon was. In the same neutral bouillon acidulated with malic acid there is growth at +10 (P_H 7.1); none at +19.5 (P_H 6.5). Acidulated with lactic acid, there is growth at +9 (P_H 7.1). There is none at +22 (P_H 6.1).

Thinking that perhaps Mr. Lewis had used beef extract and Witte's peptone instead of beef infusion and Difco peptone, tests were made using beef extract media with these acids and with tartaric acid added to the list.

There was growth in the extract (uncorrected +7) made neutral and then acidulated with malic acid to +10 (P_H 6.2); no growth in +21 (P_H 4.9).

With lactic acid there was growth in +9 (P_H 6.4); none in +18 (P_H 4.9).

With tartaric acid there was growth in +9 (P_H 6.4); none in +20 (P_H 4.7).

With hydrochloric acid there was no growth at +21 (P_H 4.0).

In peptonized beef juice made alkaline with sodium hydroxid, growth takes place to −6 (P_H 8.7). No growth takes place at −9 (P_H 9.1).

With peptonized extract of beef, the titration of which was +7 reduced with sodium hydroxid to −2, −6.5, and −13.5, there was growth at −2 and −6.5 (P_H 8.4 and 9.4, respectively).

In a long series of tests with peptone-beef bouillon in which the beef juice was used, the titration range of the organism was found to be −6 to +28 and the P_H range 8.7 to 5.7.

Mr. Lewis's notes are as follows regarding sensitiveness to acids and alkalies:

> The organism grows best in culture media that are acidulated to +15 or +20 with HCl. Growth does not occur at +40 and is retarded above +30. The same degree of acidity is not tolerated when malic, tartaric or lactic acids are employed. The growth in +8.5 lactic acid appears to be about the optimum for this acid, and the maximum is lower than for hydrochloric. Growth fails at +30 tartaric and malic.
>
> In media titrated to −10 with normal NaOH the growth is retarded, while no growth occurs at −20. In neutral bouillon growth proceeds more slowly than in +10 or +20.

MORPHOLOGY OF THE ORGANISM

The organism was studied in the cells of the spots on the leaves which had been sectioned and stained with carbol fuchsin and in 2-day-old beef agar cultures stained with carbol fuchsin. It is a short rod with rounded ends; stained in diseased material it is 0.62 μ to 1.46 μ long and 0.61 μ to 0.83 μ wide. In the agar cultures the size is 0.62 to 1.25 μ long and 0.41 μ to 1.04 μ wide. In cultures the rods hang together in chains of 2 to 16 elements, but mostly 6 to 8. Capsules were demonstrated in 2-day-old agar cultures stained with Ribbert's capsule stain and also with carbol fuchsin.

Flagella were stained with Casares-Gil's flagella stain. There is one flagellum at a pole, rarely one at each pole. One case was observed of a branched flagellum at a single pole. No spores were demonstrated in a culture of any age nor were any involution forms seen. The organism is not acid fast nor does it stain by Gram's method but is stained readily with ordinary basic aniline stains.

The morphological differences between the two organisms are slight. The size of the Lewis organism is 1.2 to 1.8 μ by 0.6 to 0.8 μ, with rare involution forms. No spores or capsules are demonstrated. It is Gram negative, not acid fast, and bears 1 to 3 polar flagella at one pole only.

NATURAL INFECTION AND CONTROL

The disease under the writer's observations is one that occurs throughout the Eastern States in greenhouses and attacks the plants usually when they are rooted cuttings and growth is being forced. We have never noticed the disease on old, slow-growing plants except in a few cases when the plant had been cured of the disease and then the spots were few and on leaves that had held over from the early attack. When the disease occurs out of doors, as it sometimes does, it is due to crowding or to unfavorable weather conditions acting on susceptible varieties. The occurrence of the disease on the grounds of the United States Department of Agriculture was mentioned in the early part of this paper. It is probable that the disease began in the greenhouse unobserved and continued when the plants were set out in the beds, hot weather and moist conditions favoring a rapid development of the disease. It has not occurred in the grounds for the last 10 years, the very susceptible varieties having been discarded.

There has never been any insect present on any of the diseased material received from the various sources. Occasionally geranium leaves are received which are infested with red spider (*Tetranychus telarius* L.), but the spotting due to this mite is of a different type.

From all the evidence gathered the organism seems to be one harbored in the soil. The disease is not a serious one unless the physical condition of the plant is weakened by too rapid growth and too moist or too warm an atmosphere with little chance of air circulating between plants, and last, but not least, too little care in watering.

Experiments leading to the control of the disease could not be undertaken in the greenhouses where the disease occurred because of distance. Control work, however, was attempted with our own plants on which the disease had been produced. The spotted leaves were picked off, the plants separated from each other in order to give them light and

plenty of air, and care was exercised in watering them. Under these conditions the disease disappeared entirely by the end of six weeks and did not return.

TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium pelargoni, n. sp.

A motile rod with rounded ends, usually borne in pairs, occurring also in chains average size 1.02 by 0.67 μ; one polar flagellum; capsules; no spores or involution forms noted; agar colonies cream colored, round, shining, with delicate internal markings; liquefies gelatin slowly; reduces litmus and methylene blue and greens the latter; produces ammonia, indol (slight) and hydrogen sulphid; does not reduce nitrates to nitrites; grows weakly in Uschinsky's and Fermi's solutions and not at all in Cohn's; coagulates sterile milk; is aerobic; has feeble diastasic action on potato starch; maximum temperature 35° C., minimum 1°, optimum 27°; thermal death point between 51° and 51.5°; resists drying six days when 6-day-old cultures are used; tolerates sodium hydroxid in peptone-beef infusion to −6 Fuller's scale (colorimetric determination P_H 8.7) and hydrochloric acid to +28 (P_H 5.7); does not produce gas from sugars or alcohols tested; is Gram negative; not acid fast; stains readily with carbol fuchsin, methylene blue, and gentian violet; is pathogenic to cultivated geraniums, causing dead spots on the leaves.

The organism is unlike the one described by Lewis from Texas as the cause of a spot disease of Erodium amd Pelargonium and may be called *Bacterium pelargoni*. It has been reported from various parts of the Eastern United States from Virginia to Massachusetts and has been under observation in the Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture, at different times for the last 20 years.

SUMMARY

A bacterial leafspot disease of the cultivated geranium occurs widespread in the Eastern States. It is mostly a greenhouse disease but occurs occasionally on plants grown out of doors.

The organism was isolated from diseased plants received from different sources and the disease reproduced on the leaves of healthy plants.

Warm, moist conditions with poor ventilation are necessary for the organism to infect the leaves extensively.

Care in regulating the temperature, air, and moisture conditions of the greenhouse and in giving plenty of space to plants grown out of doors will go far toward preventing the appearance of the disease and toward curing it when it is present. All spotted leaves should be removed and destroyed. Very sensitive varieties should be discarded.

The name *Bacterium pelargoni* is suggested for the organism causing the disease.

PLATE 1

A.—Bacterial spots occurring on plants in Department of Agriculture grounds, Washington, D. C.

B—Bacterial spots occurring on greenhouse plants in Maryland. Natural size.

A
B

PLATE 2

Geranium leafspot from New Jersey. Natural size.

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PLATE 3

A.—Geranium leaves inoculated with leafspot organism (New Jersey) by spraying. May 5, 1920. Photographed May 26, 1920.

B.—Geranium leaves sprayed with water suspension of geranium leafspot organism (Maryland) March 31, 1915. Photographed April 25, 1915.

Natural size.

A
B

HYDROGEN-ION CONCENTRATION AND VARIETAL RESISTANCE OF WHEAT TO STEMRUST AND OTHER DISEASES [1]

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INTRODUCTION

The historical development of the subject of varietal resistance to disease among plants has been traced in considerable detail by a number of writers (*4, 9, 21, 29, 31*),[3] so that a brief summary of the many theories proposed in the literature will suffice to give the background for the present study. Among the morphological characters which have been considered responsible for resistance to some parasite are size of stomata, waxiness or hairiness of leaves or stems, toughness or thickness of cell walls or cuticle, and structure and extent of root system. Among the physiological characters are the ability to form cork around the invading organism; such rapid growth of the host that the parasite is unable to keep up with the growing tip; the presence of phagocytes or antitoxins in the host (conferring immunity in a manner analogous to that of animals); the activity of enzyms; the concentration of the cell sap; the lack of any substance stimulating the chemotropic responses of the organism necessary for the latter's successful entry and progress in the susceptible variety; the lack or subminimal amount of some nutrient necessary to the growth of the parasite in the host tissue; the presence in lethal quantity of some substance toxic to the invading organism. In support of these various views comparative determinations of many plant constituents, among them silica, manganese, cellulose, starch, sugar, tannin, protein, volatile oils, and acids, have been made.

Of late years there has been considerable speculation on the probability that the concentration of free hydrogen-ions in the cell sap of the host is the determining factor in resistance. So far, no definite relation has been established between this character and degree of resistance to parasites. Hawkins and Harvey (*14*), working on potato varieties resistant and susceptible to *Pythium debaryanum*, Weiss and Harvey (*33*) on *Chrysophlyctis endobiotica* in potatoes, and Spriesterbach [4] on wheat rust, all report that hydrogen-ion concentration bears no relation to ability to resist disease. The present investigation was undertaken for the purpose of furnishing additional data on the hydrogen-ion concentration of the expressed juice of a number of wheat varieties, some resistant and others susceptible to stemrust (*Puccinia graminis tritici* Pers.).

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[2] Grateful acknowledgement is here made to Dr. H. B. Humphrey and Dr. H. Hasselbring for their helpful suggestions and criticisms. To the office of Physiological and Fermentation Investigations I am indebted for the loan of equipment.

[3] Reference is made by number (italic) to "Literature cited," 384–386.

[4] SPRIESTERBACH, D. O. BIOCHEMICAL STUDY OF RESISTANCE TO DISEASE IN PLANTS. Unpublished report referred to by Gortner (*10, p. 40*).

MATERIAL,[5] APPARATUS, AND METHODS

Most of the plants were grown in the greenhouse; but when very young material was desired the seedlings were grown to a height of 3 or 4 inches, without light, in the germinating chambers of the Seed-Testing Laboratory of the Bureau of Plant Industry. By this means, the possibility of error from soil adhering to the plants was avoided. The greenhouse plants were grown either on the benches or in pots; the germinator seedlings on clean cloths on trays in the practically saturated atmosphere of the incubators at a temperature of approximately 20° C. The varieties were always grown and studied in pairs, consisting of a resistant and a susceptible one, as similar morphologically as possible, or in groups of three or four, in order that varying environmental conditions might affect in like degree the resistant and susceptible types in any given set of determinations and thus not obscure inherited differences.

For analysis, germinator seedlings were cut off just above the seed and soil-grown seedlings 1 or 2 inches from the ground in order to avoid contamination by soil that might be clinging to the lower part of the culms. They were ground to a fine pulp in a food chopper which, being without any sharp cutting edges, macerated the material thoroughly. The juice was then squeezed from this pulp by hand through small muslin bags which had been washed and boiled thoroughly in distilled water and used but once. The juice was kept covered in glass-stoppered weighing bottles, and the determinations were made as quickly as possible in order to avoid excessive change in reaction due to oxidations. Centrifuging was not necessary.

The plants were not frozen, as recommended by Dixon and Atkins (*8*), nor otherwise treated to make the cells permeable before expressing the sap. Harvey (*12*) has shown that freezing changes the reaction of cabbage juice. It is true that André (*1*, *2*) found that the concentration of the juice of untreated tissue varies inversely with the pressure applied, and that Marie and Gatin (*18*), Dixon and Atkins (*8*), and others have shown that the first sample pressed out may be more concentrated than subsequent ones. But Harvey (*13*) points out that in a buffered plant juice minor differences in concentration do not change the hydrogen-ion measurements appreciably.

The hydrogen-ion concentrations were determined electrometrically. Potential differences were measured with a high-resistance Wolff potentiometer which could be read to 0.00001 volt. The accessory equipment consisted of a Siemens and Halske galvanometer, a Weston standard cell standardized by the Bureau of Standards, and a 2-volt storage battery in series with a Wolff resistance box as the external regulating resistance. The hydrogen electrode usually attained equilibrium in the wheat juice in 10 or 15 minutes. The final reading was taken when the potential difference had not changed by more than 0.0002 volt in five minutes. The saturated calomel electrodes [6] used were made with the usual precautions and frequently checked by the potential difference obtained with the hydrogen electrode immersed in a *M/20* potassium acid phthalate solution, P_H 3.97 (*6*).

[5] The seed for these experiments was obtained from the agricultural experiment stations at Manhattan, Kans., Akron, Colo., Moccasin, Mont., Hays, Kans., Highmore, S. Dak., St. Paul, Minn., Pullman, Wash., Moro, Oreg., Dickinson, N. Dak., and from the agronomic division of the Office of Cereal Investigations, Bureau of Plant Industry.

[6] The form of these cells was designed by Dr. R. B. Harvey.

The hydrogen electrode was a piece of No. 24 platinum wire 18 mm. long, sealed in the end of a glass tube filled with mercury. The coating of platinum black was deposited in approximately 30 seconds from a 3 per cent solution of platinum chlorid containing a trace of lead acetate. A freshly platinized electrode was used for each determination.

The hydrogen electrode vessel was a stationary, closed cylinder of 30-cc. maximum capacity, the liquid being thoroughly agitated by the hydrogen bubbling through it at the rate of two or three bubbles per second. Electrolytic hydrogen, freed from oxygen by being passed over a palladium asbestos heating coil, was used. It entered the electrode vessel through a side arm of the glass tube enclosing and supporting the hydrogen electrode, this tube being narrowed at the lower end for the length of the platinum wire constituting the electrode so that the bubbles of hydrogen washed down forcibly and broke over the end of the wire.

Most of the measurements were made at a temperature of 25° C. in a small double-walled room, equipped with pipes with circulating water for cooling, a fan, and a heating coil automatically controlled by a Harvey thermoregulator. Temperature changes were noted and taken into account in making the computations.

For calculating the P_H values from the potential differences measured Michaelis' (*20, p. 157–158*) values for the saturated calomel cell and for the temperature coefficient were used. The presentation and comparison of data are facilitated by using the simple P_H units of Sörensen (*25*) rather than the numbers indicating the normality of the solutions in terms of grams of hydrogen per liter. Throughout this work the procedure was to make at least three successive determinations of each sample, and the average of these determinations was taken to represent the reaction of the juice. Measurements on the same sample practically always agreed within 0.02 P_H.

RESULTS

HYDROGEN-ION CONCENTRATION AND VARIETAL RESISTANCE

Table I is a condensed summary of the results of the hydrogen-ion concentration determinations of the expressed juice of resistant and of susceptible varieties at various stages of development, and on which the varying environmental conditions acted in equal degree. Each P_H value given is an average of the reactions of at least four varieties.

TABLE I.—*Hydrogen-ion concentration, in P_H units, of the expressed juice of wheat varieties in different stages of development and grown in different environments*

Environment.	Age.	Averages of resistant varieties.[a]	Averages of susceptible varieties.	Approximate soil reaction.[b]
	Weeks.	P_H	P_H	P_H
Germinating chambers	1	5.96 (14)	5.98 (15)	.....
Greenhouse bench	1	5.89 (7)	5.92 (7)	7.0
Do	2–4	5.86 (14)	5.84 (14)	7.0
Greenhouse pots (unlimed)	4–6	5.89 (11)	5.87 (5)	7.0
Greenhouse pots (limed)	4–5	6.13 (7)	6.14 (4)	7.5
Field	6–7	5.54 (7)	5.54 (4)	7.0

[a] The figures in parentheses indicate the number of samples averaged.

[b] Determined colorimetrically with Wherry's (*34*) set of indicators and a set of buffered color standards, for the use of which the writer is indebted to Miss Agnes Quirk, of the Laboratory of Plant Pathology Bureau of Plant Industry.

The most evident fact revealed by the data is that there is no significant difference in the P_H values of the juice of resistant and of susceptible varieties of wheat. It is rather surprising that there is so little difference between the reactions of plants of varieties such as Kanred, Little Club, and Preston wheats, and Khapli emmer, representing not only the extremes of susceptibility and resistance to rust but also extremes of morphological characteristics. Another striking fact is that there is little variation in the reaction of the juice of the plant at different stages of growth. The averages show a slightly increased acidity of the greenhouse seedlings 2 to 4 weeks old over that of the same plants 1 week old, and but a small further increase as the plant matures. Environmental factors produce changes in reaction far greater than any due to age or variety.

Table II gives the detailed results by varieties, the grouping showing how they were paired for close comparison. Extra care was taken to grow the plants constituting each group at the same time and under identical conditions. The necessity for such precaution is illustrated by the data on germinator seedlings, which show appreciable differences between certain groups. These are not in any case to be taken as indications of varietal differences, but as indications of the effects of small differences in the conditions under which the groups were grown.

TABLE II.—*Hydrogen-ion concentration, in P_H units, of the expressed juice of some wheat varieties resistant and susceptible to stemrust*

Variety.			Age and place of growth of seedlings.					
Name.	C. I. No.	Resistant or susceptible.[a]	7 to 10 days in germinators.	1 to 7 weeks in greenhouse.	4 weeks in greenhouse, unlimed soil	4 weeks in greenhouse, limed soil.	3 months in greenhouse, limed soil.	6 to 8 weeks in field, poor growth.
Kanred	5146	R	6.01	5.87	.....	.....	.....	.....
Turkey	1558	S	6.01	5.84	5.87	6.16	.....	.....
Kanred	5146	R	5.95	5.84	.....	.....	.....	.....
Kharkov	1442	S	5.93	5.84	.....	.....	.....	.....
Pentad (D–5)	3322	R	5.99	5.87	5.81	6.08	5.88	5.61
Little Club	4066	S	5.92	5.87	5.77	6.18	.....	.....
Khapli	4013	R	5.96	5.81	5.81	6.16	5.74	5.48
Kota	5878	R	6.05	5.83	5.77	6.17	5.83	5.59
Preston	3081	S	6.08	5.79	5.72	6.12	5.76	5.53
Mindum	5296	R	5.98	5.88	.....	6.07	5.81	5.52
Arnautka	4064	S	5.98	5.87	.....	6.11	5.75	5.55
Iumillo	1736	R	.....	5.93	5.93	6.13	5.72	5.54
Average			5.99	5.85	5.81	6.13	5.78	5.55

[a] R=resistant; S=susceptible. See column 3 of Table III.

The remarkable agreement between the values obtained for varieties representing widely varying morphological types is strikingly brought out in Table II. Consideration of the figures for any group in any column, and of the results as a whole, leads to the conclusion that varietal resist-

ance to stem rust is not determined by the hydrogen-ion concentration of the cell sap.

Included in the list of wheats of which the hydrogen-ion concentration of the juices is given in Table II are varieties resistant and susceptible to diseases other than stem rust. From data kindly furnished by Dr. E. B. Mains on leaf rust, Mr. C. W. Hungerford on stripe rust, Dr. E. C. Stakman on stem rust, Dr. E. F. Gaines on stinking smut, Dr. W. H. Tisdale and Miss M. A. Griffiths on flag smut, Dr. J. G. Dickson (winter wheats) and Mr. J. J. Christensen (summer wheats) on scab, and Mr. R. W. Leukel on nematodes, together with the results of personal observations on mildew, Table III was compiled.

TABLE III.—*Resistance and susceptibility of wheat varieties to various diseases* [a]

Variety.	C. I. No.	Stem rust (*Puccinia graminis tritici*).	Leaf rust (*Puccinia triticina*).		Stripe rust (*Puccinia glumarum tritici*).		Stinking smut (*Tilletia tritici*).	Flag smut (*Urocystis tritici*).	Scab (*Gibberella saubinetii*).	Mildew (*Erysiphe graminis*).	Nematode (*Tylenchus tritici*).
		Field and greenhouse.	Field.	Greenhouse.	Field.	Greenhouse.	Field.	Field and greenhouse.	Field and greenhouse.	Field and greenhouse.	Field.
Kanred	5146	R*	R	S	S	S	R–	R	S	S	R
Turkey	1558	S*	S	–	R–	S+	R–	R	S	S	S
Kharkov	1442	S*	R–	–	R	R–	R	–	S	–	–
Pentad (D–5)	3322	R*	R	–	R	–	S	–	–	S	S
Little Club	4066	S+	S+	S+	S	S+	S+	S+	–	S+	–
Khapli	4013	R	R	R*	R–	R	R–	–	–	R	–
Kota	5878	R*	S+	S+	–	–	S+	–	R	S	S
Preston	3081	S*	S	S	R–	R	R–	–	R	S	S
Mindum	5296	R–*	R	S*	S	–	R–	–	S	R–	–
Arnautka	4064	S*	R	S*	S	S+	R–	–	–	R–	S
Iumillo	1736	R*	R	S*	S	S	–	–	–	S	–

[a] R = resistant; R– = only moderately resistant; S = susceptible; S+ = extremely susceptible; * = not susceptible nor resistant to all biologic forms.

The variable behavior of these varieties with respect to infection by the different organisms is interesting, especially since Vavilov (*31*) has come to the conclusion that if a variety is susceptible or resistant to one it is very likely to be correspondingly susceptible or resistant to all. The uniformly high susceptibility of Little Club and resistance of Khapli are the only examples in the preceding table of consistent behavior in this regard.

From the data given in Table II and Table III, we can conclude that there is no correlation between the hydrogen-ion concentration of the expressed juice and varietal resistance or susceptibility to any of these diseases. The possibility that the total acidity of the cell sap may play an important rôle in the progress of the chemical reactions between the host protoplasm and the destructive secretions of the invading fungus remains and is now being investigated; but that the concentration of free hydrogen ions is a determing factor is precluded by the data here reported.

EFFECTS OF ENVIRONMENTAL FACTORS AND EXPERIMENTAL PROCEDURE ON THE HYDROGEN-ION CONCENTRATION OF WHEAT JUICE

In the following pages are described a number of experiments designed to determine the magnitude of errors that are likely to be introduced into measurements of the hydrogen-ion concentration of plant juices,

either as a result of a summation of accidental errors or as a result of systematic errors in operation.

Table IV shows the results obtained from nine different plantings of seed of four varieties, each figure being an average of three determinations. They were grown at different times and so represent effects of the small variations in temperature, moisture, or other environmental conditions in the incubators, which often were sufficient to cause a noticeable difference in the rate of growth of the seedlings. Of course, the handling of the samples from the time the plants were cut until the reaction of the juice was determined was made as uniform as possible.

TABLE IV.—*Range in the P_H values, under the conditions of the experiments, of some wheat varieties resistant, and some susceptible, to stem rust*

Experiment No.	Kanred (resistant).	Turkey (susceptible).	Khapli (resistant).	Little Club (susceptible).
1	6.06	6.06	5.92	5.92
2	6.02	6.00	5.99	5.86
3	6.02	6.00	6.00	5.91
4	6.06	5.96	5.94	5.92
5	5.94	6.00	5.96	5.93
6	6.00	5.95	6.03	5.98
7	5.99	6.07	6.00	5.92
8	5.94	6.04	5.97	5.90
9	6.00	5.97	5.94	5.92
Average	6.00±0.010	6.01±0.010	5.97±0.008	5.92±0.007

There is a difference of a little more than 0.1 P_H between the extremes for each variety.[7] These are typical of the variations in the values obtained for any variety whether grown in the germinators or in the greenhouse.

In order to determine whether minor differences in procedure during the handling of the sample played any part in producing the range of values reported in Table IV, or whether it was due solely to the slight changes in environmental conditions, four trays of Kharkov seedlings were grown in the same germinator at the same time, one above the other. On the seventh day these were expressed successively with the following results:

Sample No.	P_H
1	5.922
2	5.921
3	5.916
4	5.921

The close agreement between these values would seem to indicate that such differences as are recorded in Table IV are to be attributed to varia-

[7] These four varieties are especially interesting from the standpoint of their resistance to stem-rust. So far as we know now, Khapli is very resistant to all biologic forms of this rust and Little Club is very susceptible to them all; Kanred is immune from certain forms to which Turkey is very susceptible. According to Table IV, Little Club has a slightly greater hydrogen-ion concentration than the other varieties, the small probable errors of the averages making the difference seem significant. Whether this difference is constant for this variety when grown under incubator conditions was not determined, as too few seed lots were available. However that may be, it is clear that the averages show no correlation of acidity with rust resistance.

tions in the environmental factors affecting growth rather than to errors in operation. In agreement with this conclusion is the statement of Richards (*24*) that—

> Experimentally considered this close connection of acidity with weather conditions gives an exceedingly small expectation of precisely similar results, even with material from the same plant. . . . Even under apparently like conditions the plants are not constant in acidity.

Therefore, for varietal comparisons, no importance can be attached to differences of 0.1 P_H between single observations when the plants are not grown at the same time and under identical conditions. Since so few determinations can be made in a single day, the necessity for averaging a considerable number of observations, if conclusions are to be drawn from small differences, is evident.

It was found that the hydrogen-ion concentration of expressed wheat juice increases on standing, which is in agreement with results reported by Clevenger (*7*) and Haas (*11*) for alfalfa and red-clover juice. Some figures showing the rate at which this gradual increase in hydrogen ions takes place in expressed wheat juice, kept in closed weighing bottles at a temperature of 25° C., may be of interest. Three separate determinations were made on the same sample at intervals during the day as indicated below.

A. From plants (Iumillo) 31 days old, grown in limed soil in greenhouse:

	P_H
a. m.	
10.33	6. 122
10.45	6. 129
11.00	6. 132
p. m.	
1.50	6. 089
2.05	6. 081
2.15	6. 071
4.10	6. 015
4.25	5. 998
4.35	5. 996

B. From plants (Kanred) 8 days old, grown in germinating chamber:

	P_H
a. m.	
9.50	5. 941
10.05	5. 944
10.20	5. 939
11.35	5. 923
11.45	5. 913
12.00	5. 913
p. m.	
2.00	5. 859
2.15	5. 849
4.00	5. 813
4.10	5. 805

These data show that among other precautions, uniform time intervals between the expression of the juice and the determination of its reaction must be observed to avoid errors due to oxidations or other changes in the juice.

Another possible source of error to be investigated was that of dilution. The surface moisture on both greenhouse and germinator plants, especially the latter, varied at different times, and elimination of the resulting slight differences in concentration seemed impossible. Hempel (*15*) states that a tenfold dilution of the sap of succulent plants produces no essential change in the acidity. Boas (*3*) found that the hydrogen-

ion concentration of potato juice showed no appreciable change upon the addition of a considerable volume of water. Harvey (*13*) reports that the addition of one volume of water changed the reaction of the juice of the tobacco plant by only 0.096 P_H. In the present study it was found that, in germinator wheat seedlings a week old, the addition of an equal volume of water increased the P_H value by 0.06, while the addition of two volumes raised it by 0.10. Greenhouse seedlings 2 to 7 weeks old were less highly buffered against dilution, since with them the addition of one volume of water produced an increase of 0.09 P_H and two volumes, 0.15 P_H. No varietal differences in this respect were found, the determinations being made with the same result with sap from Kanred, Turkey, Khapli, and Little Club. Each figure given is an average of results obtained from six or more different samples. From these determinations we conclude that the small differences in concentration of the juice caused by excess moisture on the leaves, by methods of expressing, or by evaporation during handling do not produce appreciable errors in the hydrogen-ion measurements.

In addition to the errors of manipulation affecting accuracy of measurements, those factors which influence the reaction of the cell sap in the growing plant must be considered before the validity of varietal comparisons can be fully established. It was thought possible that the geographical source of the seed may determine the plant's reactions to new environmental conditions in a sufficient degree to affect its acidity. Whenever possible, resistant varieties and susceptible varieties were obtained from the same experimental farm, but it was seldom possible to obtain Khapli and Little Club, the pair representing the most interesting extremes of resistance and susceptibility, from the same place. It seemed desirable, therefore, to determine the extent of agreement between the concentration of hydrogen-ions in seedlings from seed grown in different places. Below are given the P_H values of germinator seedlings, a week old, of Khapli emmer from seed obtained from widely separated regions.

Seed from Pullman, Wash., 1921.	Seed from Dickinson, N. Dak., 1921.	Seed from Akron, Colo., 1921.
5.92	6.03	5.94
5.99	6.00	5.96
6.00	5.97	5.94

Three lots of Kanred, two of them from the same station, gave the following results:

Akron, Colo., 1921.	Manhattan, Kans., 1920.	Manhattan, Kans., 1921.
5.99	6.06	6.01
5.93	6.02	5.99
5.94	5.94	5.98
5.95	6.00	5.98
5.94	5.99	5.97

Obviously, geographical differences in the source of the seed have not resulted in sufficient differences in the metabolic processes of the seedlings to affect the hydrogen-ion concentration of the cell sap in a significant degree. It is interesting in this connection to note that Kiessling (*17*), speaking of the resistance of barley to Helminthosporium, states that the specific behavior of varieties was independent of the conditions under which the seed had been grown.

As was to be anticipated from the reported results of previous investigations, it was found that plants cut in the morning almost invariably had a higher hydrogen-ion concentration than those cut from the same plot in the afternoon. As the result of many researches on the diurnal periodicity of the acidity of plant juices, well reviewed and confirmed by Richards (*24*) and by Clevenger (*7*), it is a well-established fact that there is a diminution of acid in many plants during the day and a gradual increase during the night until a maximum is reached just before sunrise. Clevenger (*7*) found that the hydrogen-ion concentration of the cell sap of cowpeas was most acid in the morning, decreasing toward night, and increasing after 9.30 p. m.; and Truog and Meacham (*30*) reported a corresponding change in the hydrogen-ion concentration of alfalfa. Hempel (*15*) proved that plants kept in the dark for not too long a time become more acid. In explanation may be mentioned the work of Purjewicz (*23*), Spoehr (*26*, *27*, *28*), and others who have shown that sunlight decomposes malic and other organic acids in vitro. The following figures taken from some of the data obtained during the present investigation, illustrate the magnitude of the difference in reaction between greenhouse wheat seedlings cut in the morning and those cut from the same plots in the afternoon of the same day, all other conditions being kept as nearly identical as possible.

A. Seedlings 2 weeks old, limed soil

	Cut 9 a. m.	Cut 1 p. m.
	Ph	*Ph*
Khapli	(*a*) 5.94 (*b*) 5.92	5.97
Little Club	(*a*) 6.00 (*b*) 5.97	6.04
Kanred	6.04	(*a*) 6.13 (*b*) 6.12
Turkey	6.08	(*a*) 6.16 (*b*) 6.13
Average	5.99	6.09

B. Seedlings 5 weeks old, unlimed soil

	Cut 9 a. m.	Cut 1 p. m.
	Ph	*Ph*
Khapli	5.79	5.85
Little Club	5.73	5.80
Turkey	5.85	5.99
D–5	5.79	5.82
Iumillo	5.82	5.99
Average	5.79	5.89

These data show that there is a decrease in the hydrogen-ion concentration of the cell sap between the hours of 9 a. m. and 1 p. m. This changed balance in the reactions involved in acid synthesis and decomposition is not surprising in view of the fact that they have been shown to be so dependent on light, temperature, and moisture conditions (*24*).

The effect of soil reaction on the hydrogen-ion concentration of the cell sap of plants is not always the same, judging from the observations reported in the literature. Contrary to what one would expect, Clevenger (7) found that the leaves of oats, soy beans, and cowpeas were more acid when there was lime in the soil, although the reverse was true in buckwheat. On the other hand, Kappen and Zapfe (*16*) state that liming the soil had no effect on the hydrogen-ion concentration of the lupine or bush bean, measured at the time of blooming; and, likewise, Promsy (*22*) says that the absorption of acids by seedlings did not change the acidity of the sap. Truog and Meacham (*30*) found, however, that in 12 out of 16 cases, including several different crop plants, lime lowered the reaction of the cell sap. Most of the evidence Haas (*11*), obtained with many different agricultural plants, indicates that plants grown in unlimed soil have a higher hydrogen-ion concentration than those from limed soil, among these being wheat, although he found a number of plants in which the reverse was true.

All the data bearing on this question of the effect of soil reaction on cell-sap reaction obtained in the present investigation, summed up in the foregoing figures and in Tables I and II, have been consistent in showing a lower hydrogen-ion concentration and lower total acid content of the juice of wheat from limed soil as compared to that from unlimed soil. However, the acidity of the limed seedlings seems to have increased to that of the unlimed seedlings by the time the plants were three months old (Table II). The data in these tables show the degree of difference in the P_H values and indicate the necessity of eliminating the soil variable in any search for heritable varietal differences in cell-sap acidity.

Whether the greater alkalinity of plants in limed soils is directly due to the absorption of neutralizing ions from the soil, or whether it is the result of some general effect on the health and vigor of the plants, is an open question. In at least one case it was noticed that the limed plants were taller and more vigorous than the unlimed ones with lower P_H. Clevenger (7) suggested that the greater acidity of the leaves of his limed plants might be explained by assuming that the plants were healthier and metabolism more rapid, but observations on wheat made during the present investigation would indicate that the less vigorous plants are the more acid. Lack of vigor due to unfavorable conditions for growth has been invariably associated with high hydrogen-ion concentration and high total acidity. The most notable examples were winter wheats grown in the greenhouse when the temperature was too high and which had the very low P_H value of 5.5. Some 14-weeks old Kanred and Turkey wheats, stunted and drooping, were 5.48 and 5.50, respectively, while Mindum and Arnautka growing alongside, heading, and in erect, vigorous growth, were 5.98 and 6.02. When, in other series, these four varieties were equally healthy in appearance, no such differences were found. It was also found that plantings of any variety on a certain shaded bench in the greenhouse resulted in slow-growing plants with lower P_H values than those from the other benches. It is very interesting to note that Truog and Meacham (*30*) raise the question as to whether the more acid reaction of lupine in limed soil might not be

due to the fact that liming sometimes injures this species. Also, some irregular results obtained by Haas (*11*) with plants grown in Plainsfield sand, in which the growth of some plants was found to be unfavorably affected by lime, might well be due to the fact that poor growth is associated with acid accumulation.

In the last column of Table II are given the P_H values of a number of varieties grown in the virgin soil of a cleared plot in a woodland (Cabin John, Md.). The weather during their growth was very unfavorable, being warm and humid, and the plants when 6 weeks old were far from vigorous. The winter varieties, Kanred, Turkey, and Kharkov, grew so poorly and were so badly infected by Erysiphe that no determinations were made on them. The high acidity of these samples was very interesting because the P_H values agreed closely with the results obtained from unhealthy greenhouse plants. It is interesting to note that Spriesterbach (unpublished report) found that frost caused a definite increase in the hydrogen-ion concentration of wheat plants.

Fungous infection, often present in these cases, was mostly mildew and leaf rust, but the acidity measurements bore no relation, as a rule, to the presence or the degree of infection, so that the acid accumulation was attributed usually to physiological derangements of some of the metabolic processes. Wagner (*32*), however, claims that the diminution of hydrogen-ion concentration immediately following the injection of phytopathogenic bacteria is followed by a steadily increasing acidity until the end of the incubation period, falling back to normal if the plant successfully resists infection. Mazé (*19*) says that fungi are active destroyers of organic acid, and Boas (*3*) says that the acidity of potatoes affected by the leaf-roll disease is less than that of healthy plants. However, Harvey (*13*) found that the hydrogen-ion concentration of tobacco plants affected with the mosaic disease was somewhat greater than that of the healthy plants, and Weiss and Harvey (*33*) state that the potato-wart organism caused an increased acidity (P_H 6.00) of the diseased tissue as compared to the healthy tissue (P_H 6.49).

In some of the writer's sowings it was found that wheat plants badly infected with *Erysiphe graminis* were abnormally acid, and it seemed possible that pathological trouble was causal. This was quite clearly indicated by Little Club, which was most susceptible to Erysiphe, yet which grew vigorously at greenhouse temperatures when free from disease. In one series, in which Kota, Preston, Pentad (D–5), Mindum, Arnautka, and Khapli, apparently clean, had P_H values averaging 5.80, Little Club, Kanred, and Turkey, badly infected, had P_H values of 5.63, 5.64, and 5.67, respectively. In such cases, however, it is impossible to say that the acid accumulation was directly due to the activities of the fungus, as it may well have been the result of decreased vigor attendant upon infection.

CONCLUSIONS

(1) It appears from the data presented in this paper that there is no correlation between the hydrogen-ion concentration of the expressed juice and the resistance or susceptibility of wheat varieties to disease.

(2) Environmental factors produce much greater differences in the hydrogen-ion concentration of the expressed juice than were ever found between varieties or between plants of different ages grown under identical conditions.

(3) The P_H value of the juice of wheat plants grown in the greenhouse averages 0.1 higher when the plants are cut at 1 p. m. than when they are cut in the morning about 9 o'clock.

(4) The hydrogen-ion concentration of the juice of wheat plants grown in limed soil is lower than that of plants grown in unlimed soil.

(5) Lack of vigor as shown by unhealthy appearance of wheat plants is always accompanied by an abnormally high acidity of the expressed juice.

(6) Plants badly infected with *Erysiphe graminis* are more acid than adjacent plants free from infection. This may be due to the poor physical condition of the plants and not to any direct effect of the presence of the fungus.

(7) The geographic source of the seed does not affect the hydrogen-ion concentration of the juice of the plants.

(8) The concentration of hydrogen ions in expressed wheat juice increases on standing.

(9) Dilution of the expressed juice decreases the concentration of hydrogen ions, the addition of two volumes of water to one of juice increasing the P_H value by 0.10 to 0.15. Young germinator seedlings are more highly buffered against dilution than older greenhouse plants.

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COTTON-WILT, A SEED-BORNE DISEASE [1]

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INTRODUCTION

The possibility that cotton-wilt is spread by means of infected seed has been recognized for many years, but in the previously reported experiments conducted for the purpose of determining whether or not the disease was seed borne negative results were secured. Fulton [3] discussed the possibility of the disease being carried to new localities on seed but reported that—

> The writer has made numerous attempts to secure cultures of the wilt fungus from seed taken from badly wilted plants but without success.

Gilbert [4] later attempted to introduce the wilt into uninfected soil by means of seed obtained from wilted plants. Apparent negative results were obtained in a test covering four successive years on the same piece of ground.

While working with some of the other cotton diseases in Arkansas, the writer was struck by the not infrequent occurrence of isolated wilted plants in otherwise healthy fields, and often on practically virgin land. It was often suggested by the farmers that the disease was introduced in the seed. The possibility of the disease being seed-borne is very evident to one familiar with the disease and the methods of cotton planting. As with most wilt diseases, plants die throughout the season and some live through to frost. The most severely attacked plants, or those infected earliest, die without producing mature bolls. Many wilted plants, however, produce mature bolls before dying. Usually the plant dies, leaving many bolls in various stages of maturity, some of which open or partly open during the picking season. During a period of wet weather the wilt fungus grows out to the surface of the dead terminal twigs of the diseased plants and often covers the surface with a coating of spores. Under such conditions it is hard to conceive how any of the "seed cotton" from a badly wilt-infected field could escape carrying the spores of the fungus on the lint.

In the spring of 1920 over 200 bushels of cotton seed were delinted by the concentrated sulphuric-acid method and further disinfected by soaking in 1 to 1,000 corrosive sublimate solution. Most of this seed was planted on virgin timber-land slash in Mississippi County, Ark., at a considerable distance from other cotton land. In this field of nearly 200 acres occasional wilt-infected plants appeared throughout the season. No satisfactory explanation of their occurrence other than that the

[1] Accepted for publication July 11, 1922.
[2] The writer acknowledges the assistance of Mr. R. F. Crawford in carrying on the isolation and inoculation experiments.
[3] FULTON, H. R. COTTON WILT. La. Agr. Exp. Sta. Bul. 96, 15 p., illus. 1907.
[4] GILBERT, W. W. COTTON DISEASES AND THEIR CONTROL. U. S. Dept. Agr. Farmers' Bul. 1187, 32 p., 18 fig. 1921.

organism was carried within the seed seemed possible. In 1921 in a small plot of cotton grown at Fayetteville, from thoroughly surface disinfected seed, two wilt plants appeared. The plot was far from the cotton area and had never before been planted to cotton. One of the two diseased plants died early, producing no bolls; the other lived throughout the season. These two cases strongly suggested the possibility that the cotton-wilt fungus may be carried on the inside of the seed coat, and the experiments here reported were directed primarily toward discovering whether or not such was the case.

METHODS OF PROCEDURE

Seed was carefully selected from wilt-infected cotton plants, delinted with concentrated sulphuric acid as a preliminary treatment, then further disinfected before planting. The seed was germinated under aseptic conditions, and as far as possible all fungi appearing on either viable or dead seed were identified. Fungi which from their morphology and their biochemical reactions were judged to be *Fusarium vasinfectum* Atk. were saved for further tests expected to establish their identity. No fungus was definitely accepted as being *F. vasinfectum* until successful inoculations of cotton plants had been secured. At first the plants which had died, apparently of wilt, were strongly surface sterilized, and the fungus was reisolated from all parts of the stem; but in the later tests the typical wilting, accompanied by the blackening of the xylem, was considered final proof of the identity of organism as *F. vasinfectum*.

ISOLATION OF THE FUNGUS

Seed from badly wilted cotton plants, in which the vascular infection could be traced into the bolls from which the seed was taken, was selected in the field September 21 to 24, 1921. The selections were made with particular care, which fact probably had much to do with the results subsequently obtained. After the longer lint had been hand picked from the seed, the seed was delinted with concentrated sulphuric acid, washed and dried, and put away in a cloth bag until used for plating. Each lot of seed was again subjected to about five minutes' treatment with concentrated sulphuric acid, washed, treated for two minutes in 1 to 1,000 mercuric-chlorid solution, and washed in sterile water before plating. It would seem impossible for any organism external to the seed coat to survive this treatment, and all organisms isolated from seed so treated have been considered as coming from inside the seed coat.

Plating was begun September 27, three days after the seed was gathered. Four series of platings were made within a month of the time the seed was gathered, and the wilt fungus was isolated 31 times from 524 seeds, or from practically 6 per cent of the seeds plated. Within three months from the time the seed was gathered, the fungus had been isolated from 39 of 769 seeds plated. The first plating was made on cornmeal agar, but in all following series the plating was done on white blotting paper in Petri dishes sterilized in an autoclave. All fungi appearing on the seeds were saved for identification, at first by transferring them to corn or potato agar stants, later by transferring the suspected fungi to rice tubes for the color reaction of the fungi, which in the case of the wilt organism is rose pink, or a little darker. Germinated seeds which showed

no indication of disease were transferred to test tubes of corn meal or potato agar, originally with the intention of using them for inoculation tests, but, as several of these seedlings developed signs of wilt, they were saved in later series for the purpose of observing possible development of signs of wilt infection. Nearly half of the total isolations of the wilt fungus were secured by this method (Pl. 2, A).

After the definite identification of some of the fungi isolated from the cotton seed as *Fusarium vasinfectum*, the remainder of the seed was saved for plating at intervals of about one month, for the purpose of determining the length of time the fungus may remain viable within the seed.

The isolation series is given in Table I.

TABLE I.—*Isolations of Fusarium vasinfectum from cotton seed.*

Date isolated.	Series.	Number of seeds.	Number of isolations of *F. vasinfectum*.	Viable infected seed.[1]	Viable seed not infected.[1]	Dead infected seed.[1]	Dead seed not infected.[1]
Sept. 27, 1921	A	105	4	...	.....	.....	.....
Sept. 28, 1921	X	119	5 +11	2 +11	68	3	46
Oct. 6, 1921	B	150	6	...	77	.....	.....
Oct. 16, 1921	C	150	5	4	110	1	35
Oct. 25, 1921	D	150	5	4	109	1	45
Dec. 12, 1921	H	95	3	0	50	3	42
Jan. 17, 1922	K	100	1	0	35	1	54
Feb. 16, 1922	J	200	4	1	80	3	116
Mar. 14, 1922	Y	200	1	0	.....	1	.....
Apr. 17, 1922	T	200	1	0	82	1	117
Sept. 27 to Oct. 16, 1922.	F[2]	.....	11	11	.....	.....	.....
Total	.....	1,469	46	22	413	14	458

[1] The isolation of the wilt fungus only is considered here in the terms "infected" and "not infected."
[2] The F series was isolated from what at first were apparently healthy seedlings from the series A, X, B, and C, after these had grown for some time on agar in test tubes.

An examination of Table I will show that the percentage of isolations of *Fusarium vasinfectum* fell off rapidly after the third month. The fungus was recovered 39 times from 769 seeds plated within three months of the date of collecting, and only 7 times from 700 seeds plated from the fourth to the seventh month, the percentage of isolations falling from approximately 6 per cent in the first month to ½ per cent in the sixth and seventh months. The plating of April 17 exhausted the supply of selected seed. For the first six series of isolations, plump, viable-appearing seed was selected, which left a large percentage of light, immature seeds in the later series and may have affected the results to some extent, although there is no apparent reason why it should, unless the presence of the wilt fungus in the immature seed may have been more often masked by the presence of other fungi.

INOCULATION EXPERIMENTS

As has been stated above, positive inoculation results were made the final proof of the identity of the isolated organisms as *Fusarium vasinfectum* Atk. Fusaria which from their morphology were judged to be the wilt organism were used to inoculate pots of soil sterilized in an autoclave. Corn meal or cottonseed meal cultures were used in the earlier tests. Also, uninoculated meal was put in the control pots, but the meal made so favorable a medium for fungous and bacterial growth that plants in both the control and inoculated pots were killed by almost any organism which chanced to infect the meal. The use of even small quantities of corn or cottonseed meal gave these results. The toxicity to cotton seedlings of the banana-wilt fungus as reported by Brandes [6] can probably be explained as a similar case of "damping-off." This difficulty was avoided by the use of sterilized cotton stem fragments carrying the inoculum, the infected material being placed under the surface layer of soil at the time the seed was planted. Apparently healthy acid-delinted seed was used for all plantings.

The seedlings growing in soil infected with the wilt organism in some instances began to show the effects of inoculation in eight days after planting, while occasional plants lived for several weeks or months after the other plants growing in the same pots had died of wilt. There was apparently much variation in the virulence of different cultures isolated, judging from the behavior of the plants in different pots in the same series of inoculations. If the seedlings did not wilt soon after germination, they usually survived for a considerable time before showing signs of infection. No culture which had not been judged from its morphology to be of the wilt organism ever caused an infection resembling wilt, although all Fusaria obtained among the earlier isolations were tested for possible pathogenicity. The color reaction of the wilt fungus on rice also came to be considered an almost positive indication of its identity, and, in later series, no cultures were used in infection experiments which did not give the characteristic color reaction. While at first the organism was reisolated from all parts of the wilted plants, in later inoculations the wilting of the plants, coupled with the typical blackening of the xylem, was considered sufficient proof of the identity of the organism as *Fusarium vasinfectum* (Pl. 1, A). In the earlier series both uninoculated pots and pots inoculated with known wilt cultures were run as controls, but in the later series the known wilt controls were omitted. Table II summarizes the inoculation experiments.

An examination of Table II will reveal a great variation in the length of time elapsing between the planting of the seeds in the inoculated soil and the isolation of the fungus from infected plants. This may have been due to various causes. In some cases the plants died without showing positive indications of wilt but merely rotted off at the soil line. During the winter months the growing conditions were very unsatisfactory, because of poor greenhouse facilities. Apparently there was a great variation both in the virulence of the cultures and in the resistance of plants. Practically every culture has been tried out two or three times, and some have been consistently highly destructive while others have been less so. In some cases a plant or two survived a long time

[6] BRANDES, E. W. BANANA WILT. *In* Phytopathology, v. 9, p. 339–389, 5 fig., pl. 21–34 (partly col.) 1919. Literature cited, p. 388–389.

after the others had died, as in the case of the culture D 9. The pot was inoculated on November 28, the first reisolation of the wilt fungus from a wilted plant was made February 5, and the last plant shed all its leaves on March 18. This plant had a blackened xylem from the roots to the growing point of the plant (Pl. 1, B). Some of the other cultures produced rather mild cases of wilt in plants which grew for several months.

TABLE II.—*Inoculation experiments with Fusarium vasinfectum isolated from cotton seed.*

Date planted.	Series No.	Wilt reisolated.	Date planted.	Series No.	Wilt reisolated.
Nov. 28, 1921	A 9	Feb. 21, 1922	Mar. 13, 1922	H 13	Apr. 27, 1922
Do	B 1	Dec. 15, 1921	.....do	K 1	Apr. 22, 1922
Do	B 2	Dec. 23, 1921	Mar. 29, 1922	J 1	June 1, 1922
Do	B 4	Feb. 23, 1922	.....do	J 2	June 3, 1922
Do	B 9	Apr. 18, 1922	.....do	J 3	June 1, 1922
Do	C 2	Jan. 23, 1922	Apr. 24, 1922	Y 1	June 3, 1922
Do	C 4	Feb. 5, 1922	May 22, 1922	A 10	June 1, 1922
Do	C 5	Dec. 15, 1921	.....do	B 6	June 3, 1922
Do	C 10	Dec. 17, 1921	.....do	B 11	May 30, 1922
Do	C 12	Feb. 13, 1922	.....do	D 4	(1)
Do	D 7	Mar. 23, 1922	.....do	D 6	(2)
Do	D 9	Feb. 5, 1922	.....do	D 15	June 10, 1922
Do	F 1	Feb. 13, 1922	.....do	F 3	(2)
Do	F 2	Dec. 17, 1921	.....do	F 4	June 10, 1922
Do	F 5	Dec. 15, 1921	.....do	F 8	Do.
Do	F 6	May 18, 1922	.....do	F 9	June 16, 1922
Do	F 7	Dec. 30, 1921	.....do	F 10	(2)
Do	X 1	Dec. 23, 1921	.....do	F 11	June 5, 1922
Do	X 3	Dec. 15, 1921	.....do	F 12	June 7, 1922
Do	X 4	.....do	.....do	F 13	June 12, 1922
Do	X 5	Dec. 17, 1921	.....do	X 2	(2)
Mar. 13, 1922	H 6	June 3, 1922	.....do	J 4	May 31, 1922
Do	H 8	Apr. 27, 1922	.....do	T 1	June 5, 1922

[1] Pot destroyed.
[2] These cultures had not produced wilt up to Oct., 1922.

LONGEVITY OF THE WILT FUNGUS BORNE EXTERNALLY ON COTTON SEED

The isolation of the wilt fungus from surface-sterilized seed plated April 17 and its successful inoculation on cotton seedlings proved quite definitely that the cotton-wilt fungus can live over from one season to the next within the cotton seed. Another point of interest was the length of time the fungus spores would remain viable on the outside of the seed. In an effort to determine this point, on January 2 a considerable quantity of undelinted seed was quite thoroughly infected by mixing it with a large culture of the known wilt organism growing on inoculated cotton stem fragments. After thorough mixing to secure a good distribution of the spores the stem fragments were picked out. This seed was first air dried, then put away in a covered battery jar. From 50 to 100 of these seeds were plated out each month up to May 23. At every plating from 80 to 100 per cent of the seed yielded viable spores of the wilt organism. The identity of the organism isolated from this seed was determined by its morphology and color reaction and in no case by inoculations. The later platings have been made

on white blotting paper which before sterilizing was dipped in a rice-starch solution so that the color reaction of the fungus may be observed as soon as the growth of the fungus begins. The color produced on the rice-starch paper is much darker purple, more like the iodin starch reaction, than that produced on rice alone, but it is quite constant and was considered dependable in this test.

On May 3 infected seed was planted in three short rows in soil which had never before been in cotton. About 1,000 seeds were planted, and the germination was good. On May 27 a dozen or more of the seedlings from this planting were found dying and thoroughly infected with wilt. On June 9 the plants were thinned to two or three in a hill, leaving 378 plants in the plot. All plants removed were examined, by sectioning, for signs of wilt. Of the 493 plants examined, 32 gave evidence of wilt infection, making about 5 per cent of the plants showing infection up to that time.

How long the spores on the seed will remain viable in this test still remains to be determined, but it is very evident that it will live the usual period between picking time and planting. It is probable that the number of spores occurring on the artificially inoculated seed is considerably greater than is likely to occur naturally, but Edgerton[6] has shown that the number of spores of various fungi occurring naturally on cotton seed may be enormous. Under favorable conditions the number of *Fusarium vasinfectum* spores could easily be as high as reported by Edgerton for other fungi, and, under the ordinary bin storage of cotton seed, at least a considerable number should remain viable until planting time.

It would seem likely that spores of the wilt fungus might be introduced into a field without infecting the soil, or at least that several years might elapse after its introduction before the infection would become noticeable. In the light of the results obtained in the writer's work, it would seem that some such explanation must be necessary to account for Gilbert's[7] apparent negative results. In the planting of artificially infected seed mentioned above, at least 80 per cent and probably 100 per cent of the seed carried the wilt organism at the time of planting, yet only a very few plants died of wilt in the early seedling stage and those could easily have been overlooked if the plot had not been examined carefully. At the time of thinning, also, only a few of the plants having wilt showed a general infection, and many of those infected might not have given much external evidence of the disease if they had remained in the field. Undoubtedly those seeds which carry the wilt internally stand a poor chance of producing a plant that will live more than a very short time, and it is probable that an introduction of the wilt by such seed would escape notice the first year unless secondary infection of adjoining plants should take place.

It would seem that under the present system of saving cotton seed and the planting of very large quantities of seed to insure a stand, every step in cotton planting favors the introduction of the wilt disease into new fields and its general distribution in all fields. The results of the present work would seem to justify the recommendation that seed for planting purposes shall not be saved from fields badly infected with wilt. Acid

[6] EDGERTON, C. W. THE ROTS OF THE COTTON BOLL. La. Agr. Exp. Sta. Bul. 137, 113 p., 13 pl. (in text). 1912. Bibliography, p. 81-85.

[7] GILBERT, W. W. COTTON DISEASES AND THEIR CONTROL. U. S. Dept. Agr. Farmers' Bul. 1187, 32 p., 18 fig. 1921.

delinting will undoubtedly reduce the chances of introducing the disease, although organisms carried within the seed coat will escape this treatment.

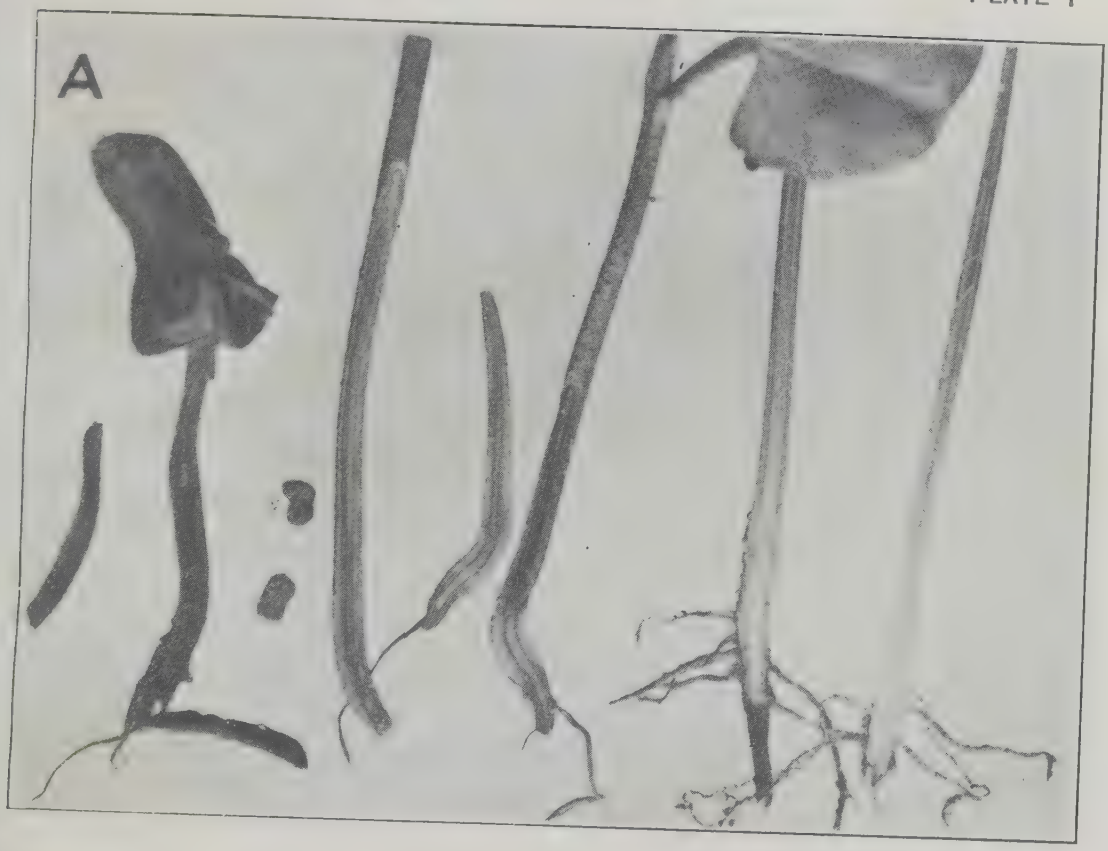
SUMMARY

The cotton wilt organism, *Fusarium vasinfectum* Atk., was isolated from strongly surface-sterilized cotton seed, indicating that the organism is at times carried on the inside of the seed coat. The pathogenicity of the organism was proved by inoculation experiments. Artificially inoculated seed carried the viable organism on the seed lint for at least five months. The wilt disease was introduced into wilt-free soil by means of artificially infected seed. It is recommended that badly infected fields be rejected as a source of seed for planting.

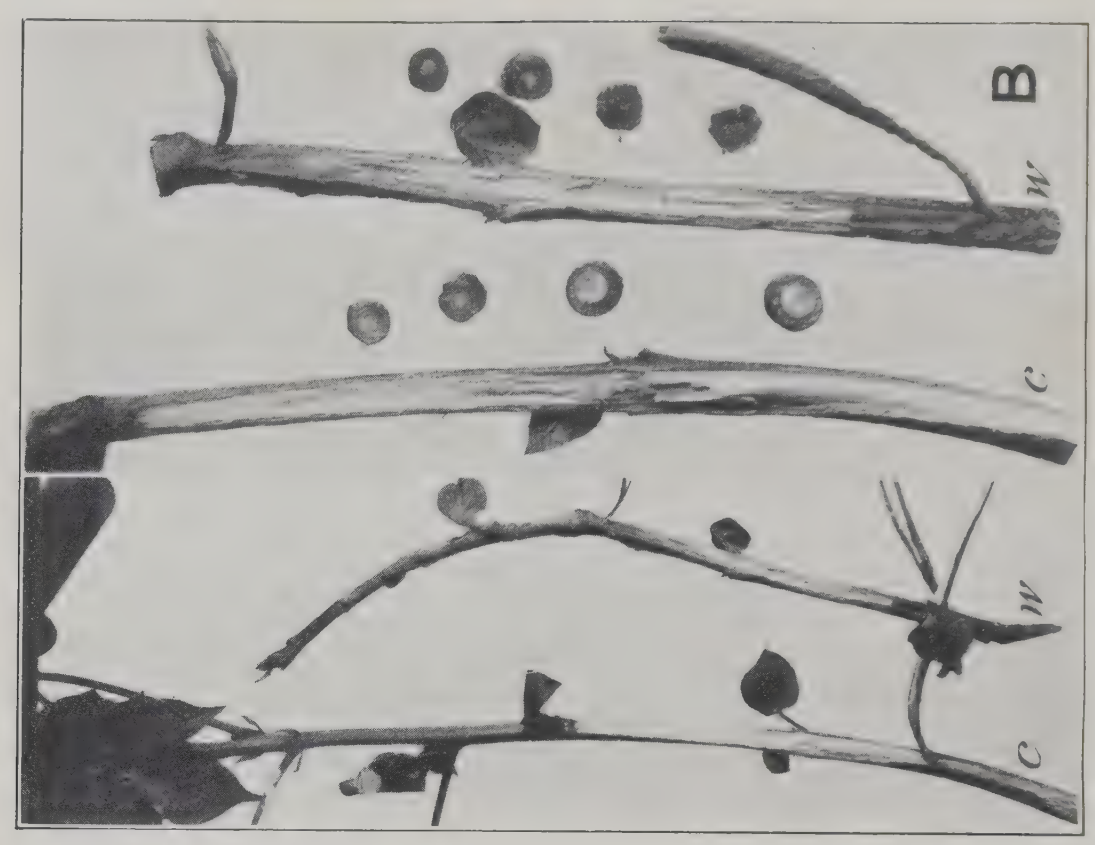
PLATE 1.

A.—Sections of healthy cotton plant from control pot on right. Sections of plants from pot inoculated with culture No. H 8 on left.

B.—Plants above in uninoculated control pots. Plants below in pots inoculated with culture No. B 1, X 3, C 5, X 4, F 5, all dying of wilt.


A

B


B
W
C
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C

A
W

PLATE 2.

A.—Four seedlings from surface-sterilized seed, transferred to agar tubes when apparently healthy. The plant in tube A was killed by *Colletotrichum gossypii*, the plant in tube W by *Fusarium vasinfectum*. The other plants were healthy.

B.—Plant W after living 5 months in pot inoculated with culture D 9. Plant C from control pot of the same series.

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IRON AND MANGANESE CONTENT OF CERTAIN SPECIES OF SEEDS [1]

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The occurrence of manganese and iron in the seeds of different species of plants has been noted by a number of investigators. However, there are but few data which show the amount of each of these elements contained in different species of seeds.

Headden [2] found that the amount of iron contained in the straw of wheat was two and one-half to six times the amount of manganese, while in the kernels the manganese is approximately equal to the iron and at the same time is higher, as a rule, than in the straw.

More recently Jones and Bullis [3] have shown that manganese is contained in considerable amounts in the aerial portions of certain leguminous plants, and from their results they conclude that alsike clover utilizes manganese in larger amounts than any other legume commonly grown in the State of Oregon and that alfalfa makes the least use of it.

Since the writer [4] has obtained data which show that manganese is a necessary nutrient in the growth of plants, it therefore becomes a matter of interest to make a determination of the amount of iron and, manganese contained in seeds of some species of plants and to correlate the results obtained.

The results contained in this paper have been obtained on samples of seeds procured from several different parts of this country. Most of the samples of wheat were obtained through the courtesy of the Kansas and Nebraska Experiment Stations and represent some of the more useful varieties grown in these and other States. A few of the samples of wheat and oats were obtained from the Departments of Plant Breeding and Farm Crops at Cornell University and had been grown on the experimental plots at that institution. Most of the other seeds were obtained from a seed company at Lexington, Ky., but the localities in which they were grown are not known to the writer.

The methods used in the estimation of the iron and manganese in the seeds were: For iron, the colorimetric thiocyanate method; [5] and for manganese, the colorimetric periodate method.[6]

[1] Accepted for publication Oct. 16, 1922. Published with the approval of the Director of the Kentucky Agricultural Experiment Station.

[2] HEADDEN, William P. OCCURRENCE OF MANGANESE IN WHEAT. *In* Jour. Agr. Research, v. 5, p. 349–355. 1915. Literature cited, p. 355.

[3] JONES, J. S., and BULLIS, D. E. MANGANESE IN COMMONLY GROWN LEGUMES. *In* Jour. Indus. and Engin. Chem., v. 13, p. 524–525. 1921.

[4] McHARGUE, J. S. THE ROLE OF MANGANESE IN PLANTS. *In* Jour. Amer. Chem. Soc., v. 44, p. 1592–1598, 1 fig. 1922.

[5] SCOTT, Wilfred W., ed. STANDARD METHODS OF CHEMICAL ANALYSIS. p. 222. 1917.

[6] WILLARD, Hobart H., and GREATHOUSE, Lucien H. THE COLORIMETRIC DETERMINATION OF MANGANESE BY OXIDATION WITH PERIODATE. *In* Jour. Amer. Chem. Soc., v. 39, p. 2366–2377. 1917. Bibliography, p. 2376–2377.

It is known that the presence of considerable phosphorus affects the accuracy of the colorimetric thiocyanate method for the estimation of iron. The following experiment was performed to determine whether the amount of phosphorus contained in seeds was enough to affect the estimation of iron by the thiocyanate method. Graduated portions of a standard solution made by dissolving 5 gm. of potassium phosphate (KH_2PO_4) in 100 cc. of water were measured out into a series of test tubes. The phosphorus contained in the different tubes varied from an amount much less than that found in seeds and gradually increased until the phosphorus content was several times this amount. To the tubes containing the different concentrations of phosphorus were added equal amounts of the necessary reagents including 2 cc. of a standard ferric iron solution, and the red color produced compared with the color developed in a tube containing an equal amount of all of the reagents used in the experiment except phosphorus.

No observable effect on the color of the solution could be detected until the phosphorus content was approximately .05 gm., an amount which is much in excess of that contained in an aliquot necessary for the estimation of iron in seeds. The color produced with thiocyanate in the presence of greater concentrations than .05 gm. of phosphorus was yellowish red instead of blood red as was produced with lower concentrations of phosphorus.

The results obtained represent the amounts of iron and manganese contained in the moisture-free seeds.

TABLE I.—*Percentages of iron and manganese found in the different species of seeds*

Variety.	Fe.	Mn.
WHEAT		
	Per cent.	*Per cent.*
Big Flame	0.0031	0.0043
Dawson	.0036	.0049
Gold Coin	.0032	.0042
Gypsy	.0051	.0049
Deitz	.0043	.0038
Nebraska No. 60	.0032	.0061
Kanred	.0039	.0036
Red Wave	.0041	.0037
Ghirka	.0040	.0073
Mutant (Nebr. No. 28)	.0042	.0043
Average	.0039	.0047
SPRING OATS		
White	0.0046	0.0058
Do	.0049	.0048
Do	.0078	.0035
Black	.0062	.0038
Burt	.0034	.0048
Rust proof	.0052	.0060
Mixed	.0034	.0052
Victory	.0042	.0052
Average	.0050	.0049

TABLE 1.—*Percentages of iron and manganese found in the different species of seeds*—Con.

	Fe.	Mn.
GARDEN PEAS		
	Per cent.	*Per cent.*
Alaska	0. 0070	0. 0012
Telephone	. 0080	. 0010
Gradus	. 0138	. 0014
Average	. 0096	. 0012
GARDEN BEANS		
Tennessee Green Pod	0. 0100	0. 0017
Kentucky Wonder (brown seeds)	. 0110	. 0016
Kentucky Wonder (white seeds)	. 0128	. 0018
Pole Lima	. 0080	. 0018
Bunch Lima	. 0100	. 0019
Average	. 0103	. 0018
SOYBEANS		
Brooks	0. 0057	0. 0021
Mandarin	. 0081	. 0025
Manchu	. 0072	. 0025
Kansas No. 1430	. 0062	. 0023
Haberlandt	. 0088	. 0033
Black Eyebrow	. 0080	. 0025
Chestnut	. 0087	. 0033
Sooty	. 0061	. 0023
Peking	. 0074	. 0041
Ito San	. 0081	. 0031
Average	. 0074	. 0028
CLOVERS		
Alsike	0. 0517	0. 0028
Red	. 0021	. 0038
White	. 0020	. 0022
White (sweet)	. 0290	. 0027
Yellow (sweet)	. 0015	. 0014
Crimson	. 0060	. 0029
Japan	. 0300	. 0155
Vetch	. 0085	. 0023
Alfalfa	. 0100	. 0012
Average	. 0156	. 0039
GRASSES		
Redtop	0. 0160	0. 0510
Timothy	. 0061	. 0072
Kentucky bluegrass	. 0350	. 0085
Orchard grass	. 0070	. 0176
Millet	. 0052	. 0017

TABLE 1.—*Percentages of iron and manganese found in the different species of seeds*—Con

GRASSES—continued

	Fe.	Mn.
	Per cent.	*Per cent.*
Corn (white H. K.)	.0017	.000
Corn (yellow)	.0026	.000
Cane (sorghum)	.0078	.001
Average	.0107	.011

MISCELLANEOUS

Sunflower	0.0034	0.002
Hemp	.0210	.016
Flax	.0085	.003
Rape	.0059	.004
Tobacco	.0240	.007

In the foregoing results the following points of interest are worthy o mention.

In the samples of wheat the average percentage of manganese is slightly greater than that of iron, which shows that the capacity of this plan for storing manganese in the seed is as great as its capacity for storing iron. This fact suggests the possibility that manganese may perform a function of equal importance to that of iron in the plant's metabolism. In one-half of the number of samples the iron was slightly greater than the manganese in the same sample. The largest percentage of manganese was found in the Ghirka variety and the largest percentage of iron in the Gypsy.

In a previous article by the writer [7] it was pointed out that manganese occurs in greatest concentrations in the outer membranes of this seed and least in the starchy, glutinous material in the interior of the endosperm. It is therefore evident that in the processes of manufacturing the highest grades of patent flour most of the manganese is removed in the offal. Then, if it should be proved that manganese is also a necessary element in the diet, it is apparent that patent flour would contain less manganese than whole wheat or graham flour.

The average results for iron and manganese in oats show that these two elements are more nearly equally distributed than in wheat.

In the preparation of oatmeal for human consumption the manganese would not be eliminated in the offal to the extent it is in the manufacture of flour from wheat. Therefore oatmeal affords one of the richest sources of manganese in food, and it is quite probable that it does add some nutritive value to this material.

The average results for iron and manganese in garden peas and beans are approximately the same in each of the different kinds of seeds. It is also of interest to note that the iron content is more than six times the amount of manganese in these seeds. The iron content in peas and beans is almost three times that found in wheat and twice the amount

[7] MCHARGUE, J. S. THE OCCURRENCE AND SIGNIFICANCE OF MANGANESE IN THE SEED COAT OF VARIOUS SEEDS. *In* Jour. Amer. Chem. Soc., v. 36, p. 2532–2536. 1914.

contained in oats. The manganese content of wheat and oats is approximately three times that contained in peas and beans.

In 10 different varieties of soybeans the average percentage of iron was nearly three times as large as the average percentage of manganese. Since soybeans have some use as a food and feed material it is of interest to note that they supply a relatively large amount of iron in the diet. Soybeans contain less iron than garden peas and beans, but more manganese.

The average results for nine varieties of clover show an iron content of nearly four times that of manganese. The average percentage of iron is about twice the iron content in soybeans, while the manganese content is about the same. Alsike clover contains nearly 20 times as much iron as manganese. While white sweet clover contains a little more than 10 times as much iron as manganese, yellow sweet clover contains nearly equal amounts of each of these elements. Both the white and yellow sweet clover seeds had been decorticated. The iron content of Japan clover is about twice that of manganese. The clovers contain considerably more iron than any of the other species of seeds examined.

In the grasses, redtop contained more than three times as much manganese as iron, while bluegrass contained more than four times as much iron as manganese. Orchard grass contained more than twice as much manganese as iron.

White and yellow field corn contained the smallest amounts of iron and manganese of any seed examined. Since most of the iron and manganese contained in corn is found in the germ and bran and since the food products of corn as they are prepared to-day exclude these parts of the grain, it is evident that such food products would be practically a negligible source of manganese.

Hemp seed contained relatively large and nearly equal amounts of iron and manganese. The largest amounts of iron were found in Alsike clover, bluegrass, Japan clover, hemp, and redtop, respectively, while the largest amounts of manganese were found in redtop, Japan clover, orchard grass, hemp, and bluegrass, respectively. Japan clover seed retains the calyx and pod, which may account for its high manganese content.

It is to be noted that the manganese content is much less than the iron content in legumes, whereas in cereals the iron and manganese contents are more nearly equal.

Seeds containing the most iron did not contain the largest amounts of manganese. In the grasses there appears to be considerable fluctuation in the iron and manganese content in one variety as compared with another. Thus manganese is in excess in redtop while iron predominates in bluegrass.

CONCLUSIONS

The average manganese content of the seeds of wheat and oats produced under natural conditions in the soil is equal to the average amount of iron found.

The amount of manganese in the seeds of legumes is much less than the iron content.

The amount of iron and manganese found in grasses showed considerable fluctuations with varieties, but the average results are nearly equal.

STUDIES UPON THE LIFE CYCLES OF THE BACTERIA—PART II: LIFE HISTORY OF AZOTOBACTER [1]

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INTRODUCTION

In a preliminary communication, published in this Journal in 1916 (*28*),[3] it was pointed out (1) that the life history of Azotobacter is much more complicated than was generally assumed and (2) that the same holds true with regard to all other bacteria. The correctness of this general statement was further demonstrated by the senior author in a critical review of the bacteriological literature, published as Part I of these Studies (*25*). It was shown therein that all findings briefly recorded in our preliminary communication are in complete agreement with numerous analogous observations made between 1838 and 1918. That these older findings had failed to lead to a more accurate knowledge of bacterial pleobiosis was caused by their being widely scattered in a gigantic literature and by their being not in accordance with time-honored, though incorrect, theories concerning bacterial monomorphism and constancy.

Since Part I was written, several contributions have been published which further support our standpoint. Bergstrand (*4*, *5*) studied the wide pleomorphism of a yellow diphtheroid and advocated that all bacteria, on account of their budding, branching, etc. should be classed among the Fungi Imperfecti. Brown and Orcutt (*7*) recorded bacillary, fusiform, filamentous branching, diphtheroid, and streptococcoid growth of *Bacterium pyogenes*. Thompson (*42*) noticed that *B. proteus*, when grown in symbiosis with *B. tuberculosis*, with other mycobacteria or actinomycetes, changed to a diphtheroid, anaerobic organism in 62 per cent of all cases. A fuso-spirillary organism was seen by Mellon (*31*) to grow (1) as a yellow actinomyces-like organism, (2) as a diphtheroid, making small semitransparent colonies, (3) as a white, aerobic, gelatine liquefying coccus, (4) as a typical anaerobic Fusiformis, and (5) as an anaerobic spirillum-like organism; filterable gonidia, "giant cocci" (evidently gonidangia) and phases of the symplastic stage were also recorded. By the same author (*32*, *33*) the "giant cocci" of *Corynebacterium Hodgkinii* were isolated and stabilized, and their "schizogony," dividing, budding and germination were carefully studied. Wade and Manalang (*44*) made an interesting report upon the various growth forms of *Bacterium influenzae*, wherein they state:

> The familiar bacillus is but a simple form of an organism capable of complex development.

[1] Accepted for publication Sept. 26, 1921.

[2] We are indebted to Mr. F. L. Goll, of the Bureau of Plant Industry, for making the photomicrographs reproduced on the plates attached to this paper.

[3] Reference is made by number (italic) to "Literature cited," p. 430–432.

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They are inclined to place it therefore among the Actinomycetes, but a comparison of their findings with those discussed in Part I of our Studies (*25*) leaves no doubt that *Bacterium influenzae*, too, follows exactly the same general lines which are recognizable in the life history of all bacteria. The general occurrence of filterable gonidia is further indicated by observations made by Heymans (*13*) with regard to anthrax and tuberculosis. It was found that the bacteria pass the filter in an ultramicroscopic form. Hort (*14*) published additional details concerning the various modes of bacterial reproduction; Howe and Hatch (*15*) confirmed Noguchi's finding that *B. bifidus* can grow as an aerobic, sporulating bacillus; and Orla-Jensen (*38*) pointed out that certain aerobic plectridia can be easily transformed so that they are no more distinguishable from the typical Proteus bacteria. Studies upon the conjunction of bacterial cells were made by Potthoff (*39*) with Chromatium and sulfur spirilla, and by Enderlein (*8*) with *Vibrio cholerae*. The results obtained are in good agreement with earlier findings (*25, p. 195, 202*). The same holds true in regard to more recent observations made by Lutz (*30a*) upon the different types of globoid bodies produced by *B. anthracis*. Almquist (*2a*) and Mellon (*33a*) have again emphasized that a thorough knowledge of the various developmental phases in the life histories of pathogenic bacteria is essential for gaining a correct view of epidemiological problems.

Our observation upon the life cycle of Azotobacter, as recorded in our preliminary paper (*28*), were partially confirmed and partially contested by D. H. Jones (*16*). Confirmed were the data on pleomorphism and symplastic stage, contested those on conjunction and on the ability of Azotobacter to assume endospore formation. The discussion of these differences of opinion will be taken up at the end of this report after our experimental results, obtained since 1916, have been presented.

METHODS OF INVESTIGATION

To obtain complete and accurate information upon the life cycles of the bacteria is no more difficult but much more time-consuming than to make an ordinary bacteriological diagnosis. Single-cell cultures and continuous microscopic observation of the living organisms are by no means so absolutely indispensable as is sometimes asserted (*25, p. 39, 205*). The generally used methods of isolating, cultivating, and studying the bacteria are, as a rule, quite sufficient to collect complete information, provided they are applied judiciously and the investigator himself is not too preoccupied by the wide-spread prejudices concerning "normal" and "abnormal" growth, "involution forms," and "contamination".

A sufficient number of parallel tests, the frequently repeated microscopic control of the cultures held for a sufficient length of time (not less than a month, preferably longer), and the regular repetition of all experiments are three points of major importance. Accordingly, apparent changes of growth can be correctly accepted as such, only if they have been ascertained repeatedly in parallel tests and if they have been closely followed under the microscope. Furthermore, an apparently new type of growth must always be tested in regard to its inclination to return to its origin, or to pass over into another well-known developmental stage of that particular organism. Sometimes the patience and persistence of the investigator is taxed very much by such experiments; it may take years before positive results are secured. A few examples in this respect were

mentioned in Part I (*25, p. 29, 34*) and others will be discussed on the following pages. On the other hand, the instability of a new form may prove vexatious. Even if a new type of growth is represented by a sufficient number of cells in a culture, and its separation by plating therefore appears to be promising, nevertheless a complete failure may result, due either to an immediate return to the original type of growth or to the disinclination of the new form to grow in pure culture on the substrate used. Occasionally the stabilization of the new form proceeds very slowly and gradually; its colonies may appear very late on the plates, or they may at first simulate those of the mother form so closely that only microscopically can a differentiation be made. However, these particular difficulties are rather an exception to the rule; usually the new type of growth will be isolated if it is represented in the original culture by a sufficient number of cells. To reduce the chances for contamination as far as possible we prefer for plating flat bottles with a neck not much wider than a test tube. The dilutions are made directly in the bottles wherein the substrate was previously sterilized.

Besides beef agar, beef gelatine, beef broth, milk, and potato the substrates most frequently used in our Azotobacter experiments were mannite-nitrate solution and mannite-nitrate agar. The composition of these media was given in our preliminary communication (*28, p. 686*). Our recipe was changed by E. R. Allen (*2*) with very unsatisfactory results; it is, however, only his substrate, not, as he asserts, the "medium of Löhnis and Smith" which proved to be unsuitable for Azotobacter growth. We studied for example, the alternation between the varied cell life and the symplastic stage of this organism in the original cultures, made in mannite-nitrate solution, for more than a year and then kept these cultures four years longer (most of the time sealed); transferred to new solution at the end of the whole period, 60 per cent of the cultures gave new vigorous growth. The hydrogen-ion concentration of these substrates was usually kept approximately at PH 6.8; occasionally it was increased to 6.0 or lowered to 7.5–8.0. Potato agar (15 gm. agar and 10 gm. peptone added to 1,000 cc. filtered potato water, prepared by boiling 200 gm. potato in 1,200 cc. water, to which an excess of $CaCO_3$ was added) was also found to be very useful, as was the case, although less frequently, with soda agar (beef agar with ¼ per cent Na_2CO_3), phosphate agar (beef agar with ⅛ per cent $Ca(H_2PO_4)_2$), salt agar (beef agar with 2 to 8 per cent NaCl), and water to which 5 cc. beef broth per 1,000 cc. were added. Most instructive results, however, were secured from cultures kept in sterilized soil (about 5 gm. in test tubes heated in the autoclave for 1½ hour at 20 pounds pressure) moistened with 0.5 per cent mannite solution. Within a few weeks all developmental stages were passed in this substrate with greatest regularity.

Some experiments upon the effect of symbiosis of Azotobacter and Radiobacter were also included. It was pointed out in an earlier paper (*26*) that especially the regeneration of large spore-free Azotobacter cells from the sporulating growth is favored by the presence of Radiobacter.

The following cultures of Azotobacter were used in our experiments:

A. chroococcum Beij. (Laboratory Numbers 1, 2, 10, 11, 12, 14, 17 to 25).

A. Beijerinckii J. G. Lipman (Laboratory Numbers 3 to 6, 13, and 15).

A. agile Beij., syn. *A. Vinelandii* J. G. Lipman (Laboratory Numbers 7 to 7c, 16 to 16c).

A. vitreum Löhnis (Laboratory Number 9).
A. sp. (Laboratory Numbers 26 and 27).

Cultures No. 1 to 11 are identical with those studied in 1914 (*26*) with the exception of No. 7b and 7c, two strains of *Azotobacter agile* recently received from Kral's Museum in Vienna (*A. Vinelandii* Lipman-Ambroz and *A. agilis* Dahlem). No. 12 and 13 are old stock cultures of the Laboratory of Soil Bacteriology. No. 14 to 16 and 16c were received from Dr. J. G. Lipman, New Brunswick, N. J.; No. 16b, 17, and 20 are subcultures of No. 112, 141, and 522 of the New York Museum of Natural History. No. 18 and 19 were received from Prof. D. H. Jones, Guelph, Ontario, in 1912 (his strains 1 and 3). No. 21 to 25 are new isolations obtained in the spring of 1916 from soil samples received from Dr. C. B. Lipman, Berkeley, Calif. No. 26 and 27 were sent to us by Dr. M. Mulvania, Knoxville, Tenn.

Fifteen of these strains (No. 7, 9, 10 to 14, 16, 16c, 18, 21 to 25) grew as typical large nonsporulating cells, when the investigations were started; 4 cultures showed only large sporulating cells (No. 2, 3, 4, and 7b), 2 small sporulating rods (No. 5 and 6), and 3 irregular fungoid cells (No. 15, 17, and 19); 4 were made up of small nonsporulating rods (No. 7c, 16b, 26, and 27), 1 of coccoids (No. 20), and 1 of dwarfed cells (No. 1). Although it was known in only some of the cases where atypical growth occurred that this was not due to contamination, we decided to include all atypical strains in our studies and to base our ultimate decision upon the outcome of these experiments.

Each of the 30 Azotobacter strains was tested in about 100 to 200 and sometimes more transfers. Each transfer was subjected to 4 to 6, but occasionally to many more microscopic tests. The results presented in this paper are therefore based on over 20,000 observations. Numerous additional tests were made with cultures of several other "species," which proved to be identical with the newly evolved growth types of Azotobacter—namely *Bacillus petasites* A.M. et Gottheil, *Bacillus malabarensis* Löhnis et Pillai, *Bacillus danicus* Löhnis et Westermann, *Bacillus pumilus* A.M. et Gottheil, *Bacillus Freudenreichii* (Miquel) Mig., *Bacillus fusiformis* A.M. et Gottheil, and *Bacterium lactis viscosum* (Adametz) Lehm. et Neum. Some of these cultures were kept in the senior author's collection; others were obtained from New York or from Vienna.

LIFE CYCLE OF AZOTOBACTER

In Figure 1 of our preliminary paper (*28*) we gave a schematic sketch of the various cell types and modes of reproduction of Azotobacter. Four types or subcycles of growth were considered to be most characteristic. The more thorough study of the problem, however, led to the discovery that from every Azotobacter culture not less than seven different growth types can be developed and stabilized; all of them are interchangeable. The cells characterizing these seven types of growth are the following (the letters in parentheses referring to the designations used in our preliminary paper):

Large non-sporulating cells (types A, B, Lα, Kα, and J).
Coccoid forms (type I).
Dwarfed cell type (types Eα, Eβ, and Kϵ).
Fungoid cell type (types G and Kγ).
Small non-sporulating rods (type Fα).
Small sporulating rods (type Fβ).
Large sporulating cells (types L, M, and Kλ).

Inspection of figure 1 of the preliminary paper (*28*) will show that the increase in the number of growth types from four to seven is due to the fact that types I and G (with Kγ) could also be stablized, and that the three types E, Fα, and Fβ could be grown separately.

In accordance with the arrangement made in Part I of these Studies (*25*) the different cell types, the various modes of reproduction, the symplastic stage, and the conjunction of vegetative and reproductive cells will be discussed consecutively. The cultural characteristics of the different developmental stages, however, will be given separately in a special chapter. The morphological features of vegetative and reproductive cells were found to be fundamentally the same with all our Azotobacter strains, while the cultural peculiarities allow a clear separation of the four species (or varieties) *Azotobacter chroococcum*, *A. Beijerinckii*, *A. agile*, and *A. vitreum*. Accordingly, the morphological facts observed with the different strains will be considered jointly, while the cultural characteristics of the four groups will be discussed separately.

I.—DIFFERENT CELL FORMS OF AZOTOBACTER

The wide pleomorphism of Azotobacter was discussed to some extent in earlier publications (*3*, *9*, *26*, *30*, and *40*). But how really bewildering the multitude of cell types is which this organism is able to produce in the course of its life cycle may be gathered from the photographs reproduced on the plates attached to this paper. Even they, of course, do not exhibit all forms which were observed in the course of our studies. But combined with the data presented in our preliminary report (*28*) and in Part I (*25*) they will suffice to indicate that an approximately complete picture of the form cycle of a bacterium is very different from the customary meager description of a so-called bacterial species.

The relatively large globules, ovals, or rodlike cells, measuring about 2 to 4 by 3 to 5 to 7μ, motile either (ovals) by polar or (rods) by peritrichous flagella, mostly Gram-negative, are in most cases very unstable. Figures 1, 2, 4, and 6 to 8 of Plate 1 demonstrate how even in young cultures (2 to 7 days old) the typical large cells are inclined to change to small globular forms (coccoids) or to more slender pointed rods, characteristic of the sporulating type of growth. In older cultures large and small rods, irregular fungoid, as well as very small dwarfed cells are always present, as is shown in figures 3, 5, 9, 11, and 12 from growths 3 weeks to 11 months old. All these forms are viable, and therefore not to be classed as involution forms. Figures 1 to 5, 9, and 11 make it fairly clear that the decrease in cell size is largely due to a loss of voluminous slimy cell elements and to the liberation and multiplication of nuclear material, which according to the quality of accessory substances presents itself as either well or weakly stainable, or as entirely unstainable when treated with aqueous dyes. Potato agar favored especially the appearance of globular cells; slightly acid mannite-nitrate agar (P_H 6.0) strengthened the tendency to produce small rods and coccoids. Only two of our cultures (No. 13 and 18) grew permanently on all substrates in the large typical form, but also in these cases the microscope always revealed the presence of the other cell types in small number.

The varying appearance and behavior of the coccoid cells of medium and of small size is illustrated on Plate 2. Figures 13 to 15 demonstrate their budding out of the larger cells; figure 16 shows multiplication by budding and by fission; figures 17, 18, and 21 represent the fairly uniform

growth of the coccoids, which were always found immotile and Gram-positive. Figures 19 and 20 illustrate the upgrowth to larger globular cells, and figures 22 to 24 the streching to small and large non-sporulating and sporulating rods. The coccoids shown in figures 17 and 18 look very much like typical micrococci, and they may, in fact, assume a great stability of growth. But sooner or later they, too, will either return to the large cell form or elongate to regular or irregular rod forms. One of our strains (coccoids of *Azotobacter agile*, No. 7) remained fairly stable as a pink "micrococcus," for not less than five years in 71 transfers, and then it returned to the large original growth type only if kept either in milk or in mannite-nitrate solution for one to two months. Milk and potato agar were usually most favorable for a return to the large cell type, while beef agar, beef broth, and mannite-nitrate solution induced the develpment of rodlike and fungoid forms.

The dwarfed cell type, first discovered in an old culture of *Azotobacter chroococcum* (*26*), can be evolved from Azotobacter cultures as easily as or even more easily than the occcoid growth. It is either immotile and Gram-negative or (the smallest units) motile and Gram-positive. The liberation of these smallest specks of mostly nuclear material from the larger cells (by budding or by so-called granular decomposition) can be seen in every culture (fig. 3 and 5 of Pl. 1). But separate development is usually slow and inconspicuous, and the isolation of this type of growth, therefore, not always easy. Pure cultures once established, however, are much inclined to reproduce larger cells, especially rods and fungoid growth. Figures 25 to 36 on Plate 3 show the typical growth and the alterations most frequently observed. A close study of the minute forms characteristic of this stage (fig. 25 and 26) reveals the tendency to present a more or less irregular, often wedge-shaped appearance. This tendency becomes more noticeable when the upgrowth takes place either by a uniting of the small granular bodies in slimy threads (fig. 28 and 29) or by their stretching to small rodlike cells (fig. 30 and 31). On the other hand, very regular, weakly staining small ovals may appear, or the small irregular cells may assume globular shape (fig. 27), simulating in this case small micrococci. The latter process is always ascertainable in cultures several weeks old, whose miscroscopic appearance is very similar to that shown in figure 18 on Plate 2. Large rod-shaped, threadlike, or large globular cells are comparatively rare in cultures of this type; if they appear they are probably always produced by the symplasm (fig. 32 to 36). The simultaneous reproduction of small cells, of long slimy threads, which are occasionally branched, and of large ovals and globules deserve special attention (fig. 33 and 36; both pictures made from the same slide). The gradual upgrowth of rods from small to large size (shown in fig. 34) is equally of great interest. The pale, small ovals, mentioned above, seem to be able to assume directly the qualities of an endospore, able to reproduce a fairly large sporulating rod. This transformation was repeatedly observed with our cultures No. 1 and 2.

The fungoid cell type is represented by figures 37 to 48 on Plate 4. Figures 37 to 39 illustrate that this irregular cell type may in fact assume distinctly fungoid appearance; figure 11 on Pl. 1 deserves also renewed inspection. Figures 37 and 38 picture the cells most frequent and most typical; they are made up of unstainable slime and darkly staining granules of nuclear material. Temporarily this slime is soluble in boiling water, and therefore the fungoid type of growth was not recognized as such in our preliminary report (*28*). But from the unstainable

granulated slime threads of the dwarfed cell type shown in figures 28 and 29 on Plate 3, the development proceeds over the steps illustrated by figures 37 to 39 on Plate 4 further to a very stable type of irregular, fungoid growth, which ultimately acquires all the qualities characteristic of a Mycobacterium. Wedge-shaped cells, budding and branching, club formation, etc. are all noticeable, and the same holds true concerning the cultural features of this "genus". The clubs again are no "involution forms," but steps in the development of rods with terminal spores (fig. 45). Dwarfed cells, coccoid forms, rods of various size, as well as large non-sporulating globules and ovals were all evolved from this type of growth, as illustrated by figures 40 to 48 (Pl. 4). Cultivation on potato was found useful for stimulating the tendency to produce clubs and rudimentary endospores. Potato agar favored again the development of coccoid forms. In water, broth, and milk branching was very pronounced, but continued cultivation in milk established a growth of regular spore-free, slime-producing rods. Alkaline mannite-nitrate solution and agar (P_H 7.5) proved helpful for reestablishing the typical Azotobacter growth. It is especially noteworthy that this was secured on these substrates with one of our cultures of *Azotobacter Beijerinckii* (No. 15) which had grown for six years in 145 transfers on practically every substrate as a highly pleomorphous mycobacterium. It had been plated repeatedly, strains representing the dwarfed growth, coccoid growth, sporulating and non-sporulating rods had been branched off, until ultimately after a passage on potato agar (fig. 44) the restoration of the original Azotobacter type was effected (fig. 47).

The typical form of the small spore-free rods of *Azotobacter chroococcum* and *A. Beijerinckii* is shown in figures 49 and 50 on Plate 5, while those of *A. agile* and *A. vitreum* are usually of more slender shape, similar to that type of growth of *A. chroococcum* visible in figure 51; fairly typical rods of *A. agile* are also to be found in the left upper corner of figure 60 on Plate 5. All these rods are Gram-negative, those of *A. chroococcum* and *A. Beijerinckii* immotile, those of *A. agile* and *A. vitreum* motile by usually three polar flagella. It should be noted that to start the cultures reproduced in figures 49 and 50 the same inoculation material was used; the difference in the substrates was the only cause of the very marked difference in appearance. A comparison of figure 49 with figure 9 and figure 24 will demonstrate the gradual changes from the large to the small non-sporulating cells, while the small coccobacilli in figure 50 should be compared on the one side with the dwarfed growth shown in figures 25 and 26 on Plate 3 and on the other hand with the short rod forms which always accompany the mycobacterium type, as demonstrated in figures 40 to 45 on Plate 4. The larger rod form producing a short branch, which is visible in the lower right corner of figure 50, is also of interest in this connection. A direct transformation from the original cultures of *A. chroococcum* and *A. Beijerinckii* to small non-sporulating rods was recorded only three times, while they were more frequently derived from the fungoid growth (nine cases observed). *A. agile* and *A. vitreum* behaved differently; several changes from the large cells to small rods, but no direct transformation from the fungoid growth could be ascertained. Figures 51 to 53 on Plate 5 demonstrate the transformation from the short non-sporulating rods to coccoid and fungoid growths, the latter identical with that of figures 43 and 45 on Plate 4. That sporulating bacilli which have lost their ability to

produce regular endospores may display a very similar morphology may be derived from a comparative examination of figure 54 with figures 51 and 53. *Bacillus pumilus*, shown in figure 54, is identical with the sporulating rod form of *A. chroococcum*, as will be discussed presently. The return from the small rods to large globular cells is illustrated by figures 55 to 57; figure 58 pictures the transformation into the typical fungoid growth, similar to that of figures 37 and 38 on Plate 4, and in figures 59 and 60 the simultaneous upgrowth of small and large rods from the symplasm is demonstrated, figure 59 making a counterpart to figures 34 and 35 on Plate 3.

The small sporulating rods are of special interest because, when the endosporulation first becomes noticeable in an Azotobacter culture, nearly always these small rods, not the large sporulating cells, are the first ones to appear. Referring to what was said in Part I (*25, p. 137, 139*) about the transformation of terminal regenerative bodies into polar exospores and endospores, it is evident that this observation is in full agreement with all earlier findings. On the other hand, it must be pointed out that these rather fragile, Gram-negative, slender rods with large polar spores are by no means a frequent occurrence in our laboratories. To discard them lightly as "contaminations" would be entirely unjustified. Identical strains were isolated from various Azotobacter cultures in Leipzig, Washington, D. C., Urbana, Ill.; and a culture recently received from Vienna gave again the same small sporulating rod. But never in our experience, and especially not in any one of approximately 5,000 transfers made in the course of these experiments, were such sporulating small rods found as contaminations. On account of their weak growth we even lost two of them by replating; but in view of this experience it is especially interesting that in old Azotobacter cultures kept undisturbed for years on mannite-nitrate agar or in mannite-nitrate solution just these small sporulating rods were the only survivors. Figure 62 on Plate 6 shows such a strain, which should be compared with the typical picture reproduced as figure 61. Figures 63, 71, and 72, on the other hand, illustrate the tendency to pass over into large sporulating rods. That transformations from small to large sporulating cells were seen occasionally in our preliminary experiments was mentioned before (*28, p. 681*). But as the first appearance of these small sporulating rods caused considerable surprise in 1912 (*26*), so it was again the case when one of these cultures (No. 6), after having displayed a very constant behavior, ultimately in the spring of 1921, when cultivated on alkaline mannite-nitrate agar, reverted to the large-cell type and assumed gradually all the characters of the original strain (No. 3), from which it had been branched off nine years before. A tendency to change to the fungoid growth is noticeable with some of the cells in figures 64. Figures 65 to 70 illustrate certain reproductive phases to be discussed on the following pages.

The large sporulating cells are shown in figures 73, 74, 76, and 77 on Plate 7 in what may be accepted as their typical appearance. They are motile by peritrichous flagella and are Gram-positive. The tendency of the threads, very common in the cultures of this type, to make short, oval, Gram-negative cells is very pronounced. Figure 78 illustrates the analogous behavior of a thread in a spore-free Azotobacter culture. In figure 75 many branched rods and threads are visible, representing the fungoid type of the large rod form, which, however, could not be stabilized, although with *Bacillus anthracis*, *B. subtilis*, and *B. mycoides*

positive results had been obtained (*25, p. 62–64*). Figure 80 deserves special attention because it illustrates a mode of fission which has been observed by very few authors and which thus far has never been photographed. Large round bodies appear, which later split on their diameter, beginning in the center, into two large, comma-shaped bacilli so characteristic of *B. Megaterium* and related "species." As was said in Part I (*25, p. 125, 126*), Schroen has described this occurrence more than 30 years ago, and at the same time E. Klein and Dowdeswell made analogous observations with *Vibrio cholerae*. The small non-sporulating rod forms of Azotobacter seem to act occasionally in a similar manner. In figure 55 on Plate 5 an intact globule is visible and also several pairs of slightly curved, plump rods, of which especially those located near the upper edge of the photograph are very suggestive. Figure 84 on Plate 7 illustrates the return to the normal large spore-free cells (after passage of the symplastic stage). In potato cultures this reversion occurred most frequently. The cells shown in figure 79 and 81 to 83 are related to reproductive processes; they represent various types of gonidangia and sporangia.

2.—DIFFERENT MODES OF REPRODUCTION

All types of bacterial reproductive organs have been found with Azotobacter—namely, gonidia and gonidangia, regenerative bodies (zygospores, etc.), arthrospores, microcysts, endospores, and exospores. It was pointed out in Part I (*25, p. 119–143*) that all these organs of reproduction are fundamentally not so different as might be assumed. As the pleomorphism of the vegetative bacterial cells finds its explanation in the varying participation of nuclear material and of other cell elements in cell construction, so also in the various modes of reproduction nuclear substances always play the dominant rôle, supported to a smaller or larger extent by reserve material, cell membrane, etc. When the nuclear material is accompanied by very little other cell elements, gonidia are produced. More reserve material and a more or less durable membrane characterize the regenerative bodies and arthrospores. When the whole cell assumes a globular or oval shape and thickens its membrane a microcyst results, while the contraction of most of the cell content and the formation of an exceptionally tough membrane leads to the formation of an endospore, or of an exospore if the growing spore buds out of the mother cell. Gonidia and regenerative bodies may multiply as such, producing the dwarfed and the coccoid growths discussed above; arthrospores and microcysts, endospores and exospores are true resting forms, although a secondary transformation to regenerative bodies or to gonidia was also observed repeatedly with these types of reproductive organs. While bacteria of the usual small dimensions as a rule form only one to two to four gonidia in each cell, the larger cells, for which the term gonidangia was introduced in Part I (*25, p. 121*) may contain a much larger number or in their place two to three or more endospores; in the latter case the gonidangium becomes a true "sporangium."

Figures 85 to 87 on Plate 8 demonstrate formation and liberation of the gonidia by normal large globular Azotobacter cells, while figure 77 Plate 7 illustrates the analogous process with the large threads of the large sporulating type. A comparison of figures 86 and 87 shows how either (in the latter case) the nuclear material practically alone may persist and give rise to the dwarfed growth discussed above, or (in the former case) how other cell elements may participate in the

regeneration of larger, in this case of coccoid cells. (See also figure 5 on Plate A of our preliminary paper, (*28*) where both processes became visible simultaneously, and figures 3 and 4 on Plate 1 of the present paper.) In figure 77 on Plate 7 the two granulated threads in the lower left part of the field deserve special attention, because the regular arrangement of the unstained gonidia contained therein is very characteristic of Azotobacter, as is the stainability of the same bodies as soon as they have left their mother cell. (See especially the dark bodies budding from several threads in figure 77 and analogous occurrences photographed in figure 60 on Plate 5.) Other types of budding, as well as the transformation of regenerative bodies into larger cells, are pictured in figures 13 to 16, 22 to 24 on Plate 2. Figure 65 on Plate 6 demonstrates the formation of gonidia in the small sporulating cells, and also their development to regenerative bodies and to endospores.

Typical gonidangia of Azotobacter may be seen in figures 5 and 11 of Plate 1 and in figures 88 to 92 on Plate 8, those of the sporulating cell type in figures 79, 81, and 82 on Plate 7; in the latter case the swollen ovals are replacing the sporulating cells, while in the two former instances the globular, branched, or spindle-shaped cells are acting as true sporangia. If small cells swell up to gonidangia they look much like regular Azotobacter cells (fig. 20 on Pl. 2 and 57, on Pl. 5), and as the latter are always able to produce numerous gonidia, it seems as if the true physiological reason for the occurrence of this developmental stage lies in the ability of these cells to act as gonidangia. The peculiarity of all bacterial gonidangia to assume either globular, oval, club-shaped, or thread-like forms is also characteristic of the large spore-free cells of Azotobacter. That they frequently multiply as such without acting as reproductive organs places them parallel to the dwarfed growth (of the gonidia) and to the coccoid growth (of the regenerative bodies). Instead of gonidia or endospores new cells of normal size may be directly formed within these large receptacles. Figures 6 on Plate A and 21 on Plate D of our preliminary paper (*28*) illustrated such cases; similar preparates are shown in figures 90 to 92 and 94 on Plate 8 of the present paper.

The fungoid type of growth was shown to be the result of a consolidation of slime threads containing numerous gonidial bodies. Accordingly it is easy to understand why in this case the formation of arthrospores is very common. The gonidia simply increase somewhat in size at the cost of the cell and surround themselves separately with cell walls. Occasionally, but much more rarely, normal rods or threads act in an analogous manner. Figures 51, 54, and 55 on Plate 5, and figure 66 on Plate 6 illustrate the arthrospore formation of the small non-sporulating and sporulating growth types. An upgrowth of other globular cells from the encapsulated symplasm also visible in figure 66 will be discussed later. The fragmentation of the large threads shown in figure 78 on Plate 7 could also be accepted as another instance of arthrospore formation, but as the globular cells produced in this case are able to multiply as such, this occurrence is to be considered more similar to that pictured in figure 21 on Plate D of our preliminary paper (*28*) and mentioned above.

Terminal swellings—that is, regenerative bodies in a polar position, but still inclosed in the mother cell—are the first step toward the formation of endospores, as was fully discussed in Part I (*25*) and frequently confirmed in the course of these investigations. Figures 45 and 46 on Plate 4,

52 to 54 on Plate 5, as well as figure 83 on Plate 7, represent the characacteristic picture of cultures inclined to begin or to resume endosporulation. Heating of such strains first to 70° C., later to 75°–90° C., provided that the tendency to produce such terminal bodies could be stabilized, leads eventually to the production of typical polar endospores or exospores. Negative results are frequent in such experiments, but they do not disprove the less numerous positive ones. As was pointed out before, these newly evolved small sporulating strains often grow very slowly and weakly, a fact which also deserves full attention. When completely developed, the endospores of the small-cell type withstood 95° C. for 4 to 5 minutes, 98° C. for 1 minute, and those of the large rods 98° C. for 2 to 4 minutes; the germination was mostly polar in both cases. Budding exospores were most clearly visible with the large sporulating rods, as was illustrated by figure 20 on Plate D of our preliminary paper (*28*). The spores do not always germinate directly; sometimes they first swell up to fairly large weakly staining ovals which are indistinguishable from microcysts. On the other hand it is no rare occurrence, especially when the small sporulating rods are cultivated in broth or milk, that the normally produced spores do not germinate but reproduce, either by budding or by dividing, 2 to 4 gonidia or regenerative bodies, which again may multiply as such (fig. 70 on Pl. 6). As was pointed out in Part I (*25, p. 141*), similar observaions were made before; they demonstrate anew that the formation of gonidia is in fact the fundamental mode of bacterial reproduction.

The transformation of vegetative cells to microcysts is most conspicuous with the large-cell types. The large sporulating rods produce weakly staining ovals (sometimes discarded as "shadows") which germinate readily to darkly staining, short, broad, mostly pointed rods (fig. 74 on Pl. 7). With the large spore-free cell type the microcysts are equally common; here they are the well-known thick-walled cells of globular, oval, or pointed shape, frequently united to two or four, whose germination was carefully studied by Prazmowski (*40*) and others. That the germ may be of coccoid or of threadlike shape is illustrated by figures 93 and 94 on Plate 8. With the small-cell types microcysts are less conspicuous. The coccoids are either thin-walled or thick-walled; in the latter case they may act as microcysts. With the dwarfed growth small pale ovals are not infrequent, perhaps a counterpart to those of the large sporulating cells. The large microcysts of the spore-free as well as of the sporulating cell type were found to be able to become gonidangia, as was to be expected.

3.—Formation of the symplasm and the regeneration of cells

In figures 7 to 18 on Plates B and C of our preliminary paper (*28*) the gradual dissolution of Azotobacter cells and the regeneration of various types of cells was first illustrated. Other pictures were published in Part I of these Studies (*25, Pls. XVIII to XXI*), here together with analogous findings of other authors. On Plate 9 of the present paper some additional photographs are reproduced, all pertaining to the symplastic stage of Azotobacter. Figures 21 (Pl. 2), 33 to 36 (Pl. 3), 38, 47, and 48 (Pl. 4), 56, 59, and 60 (Pl. 5), 66, 71, and 72 (Pl. 6), and 84 (Pl. 7) are also of interest in this connection.

All growth types of Azotobacter, vegetative as well as reproductive cells, were seen to enter the symplastic stage regularly. If vigorously growing vegetative cells were transferred, practically without exception

and on all substrates used the dissolution took place after 10 to 20 days, and about a week later the regeneration became general. If old material is transferred, typical symplasm or regeneration of cells may be seen, of course, from the start. If the transfers are repeated at short intervals the characteristic change between cellular and amorphous stage may occur much more frequently. For instance, transfers were made from a young culture of normal spore-free Azotobacter cells on mannite-nitrate agar and repeated each morning and each evening for several weeks. For a few days normal development took place, the next 6 to 8 transfers gave only very little dewlike transparent slimy growth made up of typical symplasm, later normal development became again visible, and so on in regular alternation. The tubes which first showed only symplasm gave normal growth after 2 to 3 days' delay, while with transfers made from vegetative cells no lag was noticeable (*25, p. 189*). Occasionally symplasm was also found in young colonies. On the other hand, cultures kept without change in mannite-nitrate solution for several years exhibited the regular alternation between symplastic and cellular stage quite clearly as long as the microscopic tests were continued.

As was described in our preliminary paper (*28*), the newly formed symplasm may be either homogeneous and not stainable with aqueous dyes or of a more or less hairy structure and easily stainable. These differences may persist until the new cells are formed, or changes from one to the other type may occur. Amoeboid motility, recorded by several authors (*25, p. 183*), was never observed, but strong inner movements in the amorphous clumps could be easily seen in the hanging drop. Encapsulated symplasm, as was described by Lankester in 1876 (*25, p. 170*), was only found in very few instances (in some, but not in all, milk cultures of the small sporulating rods). Figure 66 on Plate 6 shows three such "macroplasts," as they were called by the British author, intact but of relatively small size (most of them were twice as large as those photographed), and one which has liberated its sarcina-like mass of small globular cells, which on account of its thickness could not be properly focused in the picture. The streptococcus-like chains also visible are the next step in the development which led to a uniform coccoid growth of typical regenerative bodies. The regenerative units which first become visible in the symplasm may either gradually increase in size, as illustrated by figures 98 to 100 on Plate 9, or may agglomerate to full-sized cells (figs. 56 and 59 on Pl. 5; fig. 84 on Pl. 7; figs. 103 and 106 on Pl. 9). The regeneration of small sporulating rods is shown in figure 102; it should be compared with figure 15 on Plate C of our preliminary paper (*28*). Figure 104 illustrates the possibility of the newly-formed rods growing in a radiate arrangement, while figure 105 indicates that in other cases the new cells may appear along and parallel to the edges of the lobes of the symplasm. If the lobes are torn apart, as easily happens in making a preparate of such growth, a picture results which very closely resembles *Bacillus pediculatus* A. Koch et Hosaeus, while the appearance of the intact symplasm of this kind is very similar to *B. vermiformis* Ward and practically identical with *Newskia ramosa* Famintzin (*34, pp. 53–55, Pl. I*). There is no doubt that a thorough study of this type of bacterial development will release these species and the so-called genus Newskia from their isolated position in the system of bacteria. The bacteria visible at the edges are not the cause of the slime, as was assumed, but it is the (slimy) symplasm which produces

these cells first at the periphery, later also in the center. The short rods visible in figure 105 became long threads, which closely followed the outlines of the lobes of the remaining slime, until this was completely converted. Irregular spirochaetoid cells, similar to those of figure 12 on Plate 1, are shown evolving from the symplastic stage in figure 107 on Plate 9. And in figure 108 queer whiplike and fungoid forms are presented which are fairly frequent in old potato cultures of the small-cell types of Azotobacter as well as of Radiobacter. They often assume much larger dimensions, in which case no clear photographic picture is obtainable and are probably identical with what was described by Winkler as "filidia" (*25, p. 175*). As, however, not always filiform but often very irregular forms are assumed by such agglomerated symplasm (fig. 21 on Pl. 2), it seems recommendable to adopt the term "sclerotia" for these comparatively solid formations. They are no "involution forms" but produce either by fragmentation or by a second passage through the symplastic stage cells of normal shape. The term "filidia," however, may be properly applied to those more or less solid large threads which often appear temporarily in the course of the regeneration of new cells from the symplasm. Figures 33 and 36 on Plate 3 illustrate this possibility, which was also discussed in Part I (*25, p. 192*).

4.—Conjunction

Conjunct cells were regularly observed in young Azotobacter cultures of large as well as of small-cell types, usually when 2 to 4 days old. In older cultures the process may repeat itself, after new cells have emerged from the symplastic stage. It is quite evident that this uniting of two or more cells at the time preceding the formation of gonidia, of regenerative bodies, or of endospores is not without physiological significance. Especially convincing in this respect is the formation of regenerative bodies at the point where two cells united; this represents the exact counterpart to the formation of zygospores among fungi and algae. Figure 30 on Plate 3 and figure 68 on Plate 6 illustrate this occurrence very clearly.

The various modes of cell union described in Part I (*25, p. 197–203*) as characteristic of bacterial conjunction, including that of free endospores, were all observed with Azotobacter. Pictures of lateral connections and bridges, as well as the uniting of large and small cells, were demonstrated by figures 1 to 3 (Pl. A) and 22 and 23 (Pl. D) of our preliminary paper (*28*). Conjunction by bridges and by beaks are also visible in figures 278 to 294 of Part I (*25*); and figures 95 and 96 on Plate 8 of the present paper illustrate a mode of cell union which again, as the making of bridges and beaks, duplicates in a striking manner certain copulative processes known from yeasts (*25, p. 199*). That the development of endospores and of gonidangia is preceded by cell conjunction is fairly clearly indicated by the pictures shown in figures 7 (Pl. 1), 61 and 69 (Pl. 6), 81 (Pl. 7), and 89 (Pl. 8). Especially spindle-shaped and triangular gonidangia are probably always the result of the uniting of two or three cells. They are produced regularly by the large sporulating cell type as well as by the fungoid growth of Azotobacter, and they make a very interesting counterpart to analogous formations observed with spirilla. (Compare especially figure 12 on Plate 1 and figure 81 on Plate 7 of this paper with figures 86 and 87 on Plate VIII of Part I (*25*).) Undoubtedly these facts would deserve

more detailed investigation; but in view of the experimental difficulties already encountered in such studies with the comparatively large yeasts, the outlook is not very promising.

DIFFERENT TYPES OF GROWTH OF AZOTOBACTER—COMPARISON WITH OTHER BACTERIA

If the seven cell types characteristic of the life cycle of Azotobacter are separated and fully stabilized, they naturally exhibit pronounced differences in physiological and especially in cultural behavior. But our experiments revealed that there may be more than one type of growth pertaining to one type of cell form, due to different pigmentation of the bacterial growth. The large nonsporulating cells may grow white, yellowish, or brown, and the large sporulating cells either white, or yellow, or brown; but in these two cases stabilization of the differently colored strains remained more or less incomplete. On the other hand, the coccoid, the dwarfed, as well as the fungoid growths of Azotobacter, may show white, yellow-orange, or red pigmentation; and all these strains could be brought to a very marked stability. The small nonsporulating rods grew usually white, more rarely yellow; and although the small sporulating rods did not exhibit any pronounced pigmentation, they, too, showed differences of growth which thus far have been accepted as sufficient for establishing different bacterial "species." But even if only the pronounced and fairly constant differences in pigmentation are taken into account, we are confronted by the truly astonishing result that Azotobacter may present itself in not less than 14 types of growth, all so different from each other that, according to the customary methods of defining bacterial species, they all would have to be accepted as separate species belonging to five or six different genera.

Before entering upon the characterization of these different types of growth of Azotobacter a summary may be given in Table I, showing how often each type of growth was observed in our experiments, how many transformations were effected in each case, and in what direction these transformations took place. Most of the dwarfed type strains grew yellow; therefore all of them are listed in one column.

These 188 transformations effected among approximately 2,000 transfers may not appear very impressive. However, several points have to be considered in this respect in order to reach a fair valuation of these results. First, it has to be kept in mind that under the microscope the seven different cell types have been seen in all cultures, although the number of cells of different shape was frequently not large enough to permit separation by plating. Secondly, it frequently happened that when plates were made the changed cells returned to the original type of growth, or their colonies were at first so similar to the others that they escaped detection. And the third point is that the new strains, once established, repeatedly proved themselves very persistent; it was mentioned above that retransformations took place occasionally not before five or more years had elapsed, and after hundreds of transfers had been made. It might be assumed that single-cell cultures should have given better results, but as pointed out before we do not share this view.

As most of our experiments were made with *Azotobacter chroococcum* and *A. Beijerinckii*, their types of growth will always be discussed first, and then the differences obtained with regard to *A. agile* and *A. vitreum*.

TABLE I.—*Types of growth and transformations observed in strains of Azotobacter*

Type of growth at beginning of experiment.	Number of transformations to different types of growth.												
	Large spore-free cells.	Coccoids.			Dwarfed growth.	Spore-free small rods.		Fungoid growth.			Sporulating rods.		Total.
		White.	Yellow.	Red.		White.	Yellow.	White.	Yellow.	Orange.	Small.	Large.	
Large spore-free cells	....	1	2	2	4	4	1	9	0	0	5	7	35
Coccoids:													
White	3	....	1	0	3	3	0	3	0	0	3	1	17
Yellow	1	2	....	0	2	3	0	0	3	1	0	0	12
Red	1	0	0	....	0	0	0	0	0	0	0	0	1
Dwarfed growth	2	1	1	0	....	2	3	1	6	1	1	0	18
Spore-free small rods:													
White	4	5	4	0	4	....	0	1	1	1	2	1	23
Yellow	0	0	0	0	1	0	....	0	2	0	0	0	3
Fungoid growth:													
White	3	5	3	0	4	5	1	....	1	1	3	1	27
Yellow	0	0	0	0	0	0	1	0	....	0	0	0	1
Orange	0	1	0	0	1	2	0	0	1	....	0	1	6
Sporulating rods:													
Small	2	3	1	1	2	2	0	4	0	1	....	4	20
Large	5	1	4	0	4	3	0	3	0	1	4	....	25
Total	21	19	16	3	25	24	6	21	14	6	18	15	188

I.—LARGE NON-SPORULATING CELLS

In another paper, published in 1908 by the senior author together with T. Westermann (*30*), a detailed description was given of all peculiarities of the large Azotobacter cells, based on a comparative study of 21 strains, to which only few additions are to be made. The observations then made upon the tendency of Azotobacter to form branched threads and to grow occasionally as a yellow or as a white sarcina have now found their explanation in the discovery of the fungoid and of the coccoid types of growth. It was also pointed out in that report that in several respects, especially in its colony formation and in its growth on potato, Azotobacter may exhibit certain traits characteristic of sporulating rods. Numerous confirmative results were obtained in this direction. Not infrequently the colonies on mannite-nitrate agar did not show the typical coarse dark gray granulation, but a more or less hairy and brownish structure, indicative of the tendency to develop the rod form. The change from whitish to yellowish to brown growth was seen with both *Azotobacter chroococcum* and *A. Beijerinckii*. In 1908 it was said that probably *A. Beijerinckii* should be classed as a variety of *A. chroococcum;* our new findings prove the correctness of this view. Especially a white strain of *A. chroococcum* (No. 10), originally isolated by Prazmowski (*40*), behaved exactly like *A. Beijerinckii*. And several typical strains of *A. chroococcum* kept for five years in mannite-nitrate solution assumed the same character—that is, they showed now little inclination to develop the typical dark brown to black color but produced a light yellow to light brown slimy growth. On the other hand, typical strains

of *A. Beijerinckii* were also found to be able to develop a dark brown or black color when they were kept for several months in tubes containing mannite-nitrate solution and partially immersed strips of filter paper. Directly above the solution the characteristic thick yellowish to brownish slime was always noticeable, but at the upper edge of the paper strips after four to six weeks a thin dark brown to black growth became visible, and the microscopic inspection revealed the fact that the cells in this case were much more solid and more uniformly stained than those making the more slimy light-colored growth. The latter are always granular and only partially stainable, as is characteristic of *A. Beijerinckii*. With *A. agile* analogous observations were made. The slimy growth was again of light brown color like that of *A. Beijerinckii*, and at the upper edge of the paper the same black development appeared in old cultures. If *A. agile* does not produce its characteristic green pigment, as frequently happens, its growth is hardly distinguishable from that of *A. Beijerinckii;* but here other types of growth as well as slight differences in morphology permit a clear differentiation, whereas there are no such differences between *A. Beijerinckii* and *A. chroococcum*. *A. vitreum* has never shown any pigmentation of its large nonsporulating cells, which practically always retained their typical globular shape. The only exception noticed thus far was discussed in another paper (*26*); the large rodlike forms seen in this case were inclined to assume endosporulation, which could not be fully established, however. The large globular cells of *A. vitreum* leave no doubt about their being gonidangia or microcysts, and their behavior and appearance is very similar to that of globular gonidangia and microcysts of *A. agile*. A comparison of figure 6 on Plate A of our preliminary communication (*28*) with figure 94 on Plate 8 of the present paper may illustrate this similarity. These large cells are Gram-negative, as is typical for the nonsporulating large cells of *A. chroococcum*, *A. Beijerinckii*, and *A. agile*, but the somewhat smaller globular cells of *A. vitreum*, which often occur in sarcina formation, are Gram-positive. In this as well as in all other respects they display the character of the coccoid growth common to all Azotobacter strains; in fact, with all of the latter cultures microscopic pictures were obtained, especially from potato agar, which looked exactly like *A. vitreum*. In the first Azotobacter paper (*30*) it was mentioned that a white sarcina was grown from *A. agile*, as was a yellow sarcina from *A. Beijerinckii*. No explanation could be given at that time; but to-day we know that it was the regenerative bodies we had before us, and we are now also able to show that *A. vitreum* may grow in other types equal to those of *A. agile*—that is, *A. vitreum* is a variety of *A. agile*, as is *A. Beijerinckii* of *A. chroococcum*. Cultivation of the large nonsporulating cells in slightly acid mannite-nitrate agar (P_H6.0) always gave during the first two weeks good typical growth with strong characteristic pigmentation; in older cultures, however, the tendency to produce small cells became very marked and was repeatedly used to great advantage. Potato agar, on the other hand, favored especially the coccoid growth, while in soil quick changes to all different cell types were observed simultaneously.

2.—COCCOID GROWTH

As was explained before, this type of growth results when regenerative bodies cease to act as such and begin to multiply by fission and by budding. It is self-evident that in the beginning such strains are often

very unstable; they either grow up to large cells or reproduce rods either by germination or by simple stretching. But if they are once definitely established (cultivation on beef or on potato agar proved best for this purpose) they may cling tenaciously to their now micrococcus-like habit of growth. White growth was most frequent (Table I, p. 415), yellow next, and pink much more rare. Changes from white to yellow and from yellow to pink were observed in some cases, in full agreement with the results recorded with Micrococci (*25, p. 31, 43*). As *A. chroococcum* as well as *A. agile* produce all three types of growth, it is easily understood why these differences in pigmentation can not be constant.

The white coccoid growth is very similar to that ascribed to *Micrococcus concentricus* Zimm. On agar smooth circular colonies are formed, which have a finely granular structure, resembling under the microscope very much those of the *Bacterium pneumoniae* Friedl. group, except that the radiate stripes are missing. On gelatine they are distinctly zoned and have lobate edges. The growth on beef agar, as well as on mannite-nitrate agar, is varying from thin dew-like to thick aerogenes-like layers. Gelatine is not or only very little liquefied. Beef broth becomes slightly turbid and has no film or ring, but heavy sediment. Milk remains unchanged, brom-cresol-purple is slightly reduced; occasionally a slow peptonization takes place, which, however, was always accompanied by a change from the coccoid to the fungoid cell type. On potato a thick, creamy yellowish grayish brownish growth, similar to that of *Bacterium aerogenes*, was observed.

The yellow coccoid growth differs from the white one practically only by the yellow pigment produced in the cells on agar, gelatine, potato, etc., and the pink growth behaves in an analogous manner, except that the growth on potato was always very scant and colorless. Gelatine liquefaction was always absent or very moderate; motility was never seen. The pink cocci gave sometimes a brown hue to gelatine as well as to potato agar. According to the customary diagnosis, these yellow and pink coccoids would have to take their places close to *Micrococcus sulfureus* Zimm. emend. Lehm. et Neum. and to *Micrococcus roseus* (Bumm) Lehm. et Neum., respectively.

Nitrogen fixation was not noticeable with these coccoid strains. That, however, occasionally positive results may be obtained is proved by the fact that a culture, once determined as *Micrococcus sulfureus* var. *tardigradus* and found to be able to assimilate free nitrogen vigorously (*27*), has shown itself to have been the vegetative growth of the regenerative bodies of a yellow large sporulating rod, which stands very close to those developed from Azotobacter.

3.—DWARFED GROWTH

As this type of growth is the result of the vegetative propagation of the gonidia, it is always at the beginning, and not infrequently for a very long time, rather inconspicuous and easily overlooked. Very small whitish circular colonies of about ½-mm. diameter, of finely granular yellow structure in the center and of white, smooth appearance at the edge, are most typical. If the growth is more vigorous, a flaky structure may become visible in the center, as with *Bacterium septicaemiae haemorrhagicae* Hueppe (*18, Table 18, VI*). It often took two weeks before the colonies became visible. Transferred to agar, the growth is again frequently very thin and dewlike, and it may remain so on all

substrates—that is, a thin, colorless slime appears on solid media, while milk is left unchanged, and in broth as well as in mannite-nitrate solution hardly any growth becomes noticeable. More frequently, however, a better development takes place after a while, and in all cases observed by us this better growth was characterized by a bright yellow coloration, with the exception of two cases where later a red pigment was produced. The microscopical aspect and the other cultural peculiarities were identical in both cases. As no such bacterial growth was fully described before, as far as we know, the necessary data may be given here.

MORPHOLOGY (PL. 3).—Typical ⅓ to ¾μ globular, oval, wedge-shaped, or rodlike cells, single, in short chains, or often in clumps, after two to four weeks mostly small cocci, ⅓ to ½μ. Changes to small rods, to coccoids, and especially to fungoid growth were frequently observed; occasionally also upgrowth from the symplasm to large spore-free and to large sporulating cells.

STAINING after Gram was mostly negative; only in some cases very small granules showed a positive reaction, while the rest of the cells remained negative.

MOTILITY was mostly absent, only when Gram-positive granules were present the cells developed motility by one polar flagellum.

CONJUNCTION was frequent, especially in two days old cultures on salt agar and in salt-broth. Zygospores were formed.

GONIDIA were produced after the cells had grown up to full size.

GONIDANGIA of threadlike, clubbed, or globular shape, the latter equal to normal Azotobacter cells, were produced especially on potato agar, on potato, in milk, and in mannite-nitrate solution. Thin needlelike outgrowth was seen occasionally.

REGENERATIVE BODIES of globular shape appeared, as a rule, after 2 to 3 weeks, especially on agar and on potato.

MICROCYSTS.—The small globular or oval cells often became thick-walled and germinated later to small, pale, thin-walled ovals.

ENDOSPORES.—Pale, small ovals were seen to transform themselves in toto to bright, heat-resistant bodies, which gave rise to typical sporulating rods.

SYMPLASM.—Nearly always small as well as large cells developed from the symplasm, the former always and in much greater number in the thinner portions close to the edge, the latter in smaller number in the inner thicker parts. Filidia and sclerotia were also produced occasionally, the latter resembling those shown in figure 108 on Plate 9.

COLONIES.—On beef agar and on mannite-nitrate agar circular, ½ to 1 mm. in diameter, whitish to yellowish, with finely granular to flaky yellowish structure, edge white, smooth. Occasionally larger, more slimy, and more whitish colonies were formed (changing to small rod type), or larger yellow circular colonies (changing to coccoid growth), or yellow brownish colonies with somewhat irregular edge (changing to fungoid growth). On beef gelatine small yellow, dense colonies with smooth, sharp edges were formed; gelatine was not liquefied.

AGAR SLANTS.—On beef agar and on potato agar a flat, bright yellow, especially greenish yellow, growth with somewhat irregular outlines is most frequent. Occasionally it becomes orange or yellow brownish (and the cells become in these cases fungoid). On mannite-nitrate agar the growth is thinner than on beef agar, often only dew-like and transparent. Phosphate agar gave a bright greenish yellow growth. The two red strains turned from whitish yellow to orange and later to brick red; their growth remained always very moderate.

BEEF GELATINE gave only meager, thin surface growth, transparent or yellow; the gelatine remained either firm or showed after three to four weeks a little bowl-shaped depression, but only very little liquefaction.

BEEF BROTH became slightly turbid, contained some yellowish (or reddish) sediment; but only in one case a loose, thin yellow film and a thin yellow ring were produced.

MILK remained always unchanged, except in those cases where the cells assumed fungoid growth. Some yellow (or red) sediment was always noticeable; occasionally also on the surface a ring and a few flakes of the same color appeared.

POTATO gave growth, as a rule, only after repeated inoculation. Then a little transparent slime appeared, which changed with well-growing strains to a fairly thick greenish yellow (or brick red) slimy layer. Occasionally it became dull and dry (changing to fungoid type), or remained slimy, but turned brown (changing to large spore-free cells).

MANNITE-NITRATE SOLUTION gave mostly only very little growth; it showed slight turbidity and some yellow sediment; in a few cases the solution itself became distinctly yellow.

NITROGEN FIXATION was not noticeable in pure cultures, but a very pronounced stimulating effect was exerted upon the formation of thick floating membranes in mixed cultures (with large spore-free as well as with fungoid cells).

Identical transparent and yellow strains of the dwarfed growth were isolated from *Azotobacter chroococcum*, *A. Beijerinckii*, *A. agile*, and *A. vitreum*. The two atypical cultures of red pigmentation came from one strain of *A. Beijerinckii*, while two other strains of this kind did not produce such growth. It can be safely said, therefore, that the various Azotobacter species and varieties did not exhibit any difference in their dwarfed growth. *Bacterium antityphosum* Almquist, discussed in Part I (*25*, p. *147*), is equally indistinguishable from our dwarfed Azotobacter growth morphologically as well as culturally; only the agglutination test would permit differentiation. Furthermore, one culture of *Bacillus pumilus* gave us a very similar, although very weak growth, and it is to be expected that in many other cases analogous results will be obtained, as soon as the bacterial gonidia and their behavior will be made the object of adequate studies.

4. FUNGOID GROWTH

As was discussed above, this type of growth is closely connected with the gonidial development. But whereas the gonidia, when they grow as such, become similar to a minute yellow Mycobacterium, the fungoid cells in the majority of cases grew as a white, fairly large Mycobacterium, which displays on potato a very characteristic dry, raised growth of pink color. Here the differentiation of *Azotobacter chroococcum* and *A. Beijerinckii* from *A. agile* and *A. vitreum* is very sharp. Identical strains of the white-pink mycobacterium were isolated from practically all our cultures of *A. chroococcum* and *A. Beijerinckii*, but never from *A. agile* or *A. vitreum*. A few mycobacterium cultures of yellow or orange color were branched off in both cases, but these were always closely connected with the yellow dwarfed growth common to all Azotobacter strains. According to the descriptions given by Lehmann and Neumann (*18*), Söhngen (*41*), and Vierling (*43*), our yellow cultures may be identified with *Mycobacterium luteum* Söhngen, and the orange ones with *Mycobacterium lacticola* Lehm. et Neum.; but we have also to confirm Vierling's findings that the pigmentation is not absolutely stable. Changes between yellow and orange were observed and occasionally also a temporary loss of pigmentation—that is, the same instabilities as were found with the dwarfed growth.

The white-pink fungoid growth, characteristic of *Azotobacter chroococcum* and *A. Beijerinckii*, is perhaps identical with *Mycobacterium album* Söhngen. But the description given by the Dutch author (*41*) is too short to permit a definite statement. As no other description of this type of growth seems to have been published, our notes may find a place here.

MORPHOLOGY (PL. 4).—Pale, irregular, slimy sheaths with dark granules are most common on mannite-nitrate agar and in mannite-nitrate solution. They are usually the first outgrowth of the symplasm and assume gradually more regular shape and stain more uniformly. On beef agar and in beef broth the pleomorphism typical of Mycobacterium is always noticeable. Figures VI and XII of Table 69 and Figure X of Table 70 in Lehmann's and Neumann's atlas (*18*) could have been made from our slides. In milk especially richly branched forms were seen; but continued cultivation in milk caused the transformation into slime producing small rods. On potato the inclination to assume endosporulation was very pronounced, while cultivation on potato agar gave either small nonsporulating rods or white or yellow coccoid growth. In soil and in

mannite-nitrate solution, especially with P_H 7.5, the tendency to reproduce large nonsporulating Azotobacter cells was sometimes very marked, and transformations were effected accordingly.

STAINING after Gram was always positive.

MOTILITY was always absent.

GONIDIA are often liberated in great numbers especially from the slimy sheaths (fig. 40 on Pl. 4) and were frequently seen to grow up to rods or to pass over to the yellow, dwarfed growth.

GONIDANGIA.—The large, slimy threads containing numerous gonidia may be accepted as such. In addition the large, globular cells, produced in soil and in mannite-nitrate solution, which later grew as regular Azotobacter, are typical gonidangia. Such globules, when developed from the symplastic stage on potato agar, quickly dissolved into heaps of small coccoid cells (regenerative bodies).

REGENERATIVE BODIES were produced regularly in lateral or in terminal position. The former were seen to reproduce small spore-free rods, while the latter were inclined to become endospores.

ARTHROSPORES and MICROCYSTS were frequent on all substrates, but most abundant in water.

ENDOSPORES were developed from the terminal regenerative bodies in five cases. When the microscopic picture was similar to that shown in figure 45 on Plate 4, the material was heated in beef broth or in mannite-nitrate solution first to 70° C., later to higher temperatures up to 90° C., and from successful tests cultures were made on potato and on beef agar. Heated material gave at first development intermediate between normal fungoid growth and small sporulating rods. Plating and repeated heating permitted final separation.

SYMPLASM produced very manifold growth (Figs. 38, 47, and 48 on Pl. 4, Figs. 100, 102, and 107 on Pl. 9), although the typical pale, irregular sheaths with dark granules appeared most frequent. The large spore-free cells originated always from the symplasm. Irregular sclerotia, like those reproduced in figure 108 on Plate 9, were also found with this type of growth.

COLONIES on mannite-nitrate agar and on beef agar were in the beginning smaller, but otherwise very similar to regular Azotobacter colonies. When the fungoid growth was more firmly established, the colonies appeared macroscopically whitish, flat, with raised center and irregular edge, microscopically brown in the center, of hairy structure, with thin, transparent, fringed edge, very similar to Figure X of Table 69 in Lehmann's and Neumann's atlas (*18*). When the tendency prevailed to assume a more regular rod-shaped growth, the colonies became circular with sharp edge and fine granulation, whereas the inclination to change to sporulating rods caused the appearance of smaller colonies with hairy structure and irregular moss-like edge, similar to small colonies of *Bacillus mesentericus*.

AGAR SLANTS.—A flat, dry, grayish or whitish growth with thin, irregular edge is typical on beef agar as well as on mannite-nitrate agar. Often white spots are scattered over the surface. If the small spore-free rods are developing a thicker slimy white or slightly pink growth appears, while the change to small sporulating rods is accompanied by a slightly yellowish color of the layer, which may show a few wrinkles. Potato agar produced a thick yellowish pinkish growth with yellow secondary colonies, from which a pure growth of yellow coccoids was branched off.

BEEF-GELATINE.—Irregular, thin, grayish or pinkish surface growth, little development in stab; no liquefaction.

BEEF BROTH.—Uniform turbidity, loose grayish film, whitish ring, heavy slimy white sediment.

MILK unchanged during the first days, then slowly peptonized, turning brown, becoming alkaline and very slimy; on the surface often a brittle film and a whitish ring are visible. After repeated transfers from milk to milk the peptonization and brown discoloration ceased and the milk remained neutral and became very ropy; the cells had changed from the fungoid type to small nonsporulating rods.

POTATO.—Typical raised, dry, pink growth with irregular surface and edge. Rarely a yellowish grayish brownish, more or less slimy growth was produced; in these cases the irregular cells assumed rod shape.

MANNITE-NITRATE SOLUTION became slightly acid, remained practically clear; on the surface a thin grayish film developed, which often ascended on the walls of the tubes and frequently showed the white dots characteristic of the growth of *Azotobacter Beijerinckii;* on the paper above the solution a thick slime developed, which later turned brownish, and a heavy, white, slimy sediment was formed in the solution.

NITROGEN FIXATION by pure cultures in mannite solution was not noticeable, but there was a pronounced tendency to produce the wrinkled dotted film, characteristic of crude Azotobacter cultures, especially when the fungoid cells grew in symbiosis with the dwarfed cells.

The characteristic pink growth on potato reminded the senior author that some strains of *Bacterium lactis viscosum*, tested by him in 1904 for nitrogen fixation, had presented a very similar appearance (*23*). One of these cultures was still available, as was another strain of the same organism, isolated in 1909 from slimy milk. When tested, both strains behaved exactly alike and appeared very similar to the white fungoid growth of Azotobacter. Cell morphology, colony formation, the characteristic growth in milk and on potato were the same in both cases, and it was on account of these observations that with the fungoid Azotobacter strains frequently repeated tests in milk were made which, as was mentioned above, caused indeed a transformation to slime-producing rods, which proved to be identical with *Bacterium lactis viscosum*.

5.—SMALL NONSPORULATING RODS

White and yellow nonsporulating rods were observed with all four types of Azotobacter; but their morphological and cultural behavior allowed a clear separation, those dervied from *Azotobacter chroococcum* and *A. Beijerinckii* being sharply differentiated from those of *A. agile* and *A. vitreum*.

The white slime-producing small rod form of *Azotobacter chroococcum* and *A. Beijerinckii* exhibited all marks of *Bacterium lactis viscosum* (*18, p. 316; 23, p. 587, 590*). A transfer from Adametz's original culture, which was received recently from Kral's Museum, differed considerably from the older descriptions, including that published by Adametz (*1*). The rods were of very irregular size and shape, motile by peritrichous flagella; they produced gas from mannite and various sugars, but milk was still made slimy. They also gave a yellow dwarfed strain, and a yellow as well as an orange fungoid growth identical to that of *A. chroococcum*. Furthermore, large globular and oval cells were formed quite readily which resembled typical Azotobacter cells very closely (fig. 48 on Pl. 4 and fig. 57 on Pl. 5). That *Bacterium lactis viscosum* is able to fix nitrogen was shown before by the senior author (*23*).

The yellow nonsporulating rods developed from *Azotobacter chroococcum* and *A. Beijerinckii* were practically counterparts to the white rods, as well as to the yellow strains branched off from *Bacterium coli*, *Bact. typhosum*, etc. (*18, p. 382; 25, p. 57*). On the other hand, they are directly related to the dwarfed yellow growth, as well as to the yellow fungoid type, from which they were developed and into which they could be transformed. Some strains were motile by polar flagella, others were immotile; all were Gram-negative. They liquefied beef gelatine to a varying degree, grew on beef agar as a slimy, bright yellow layer, made milk alkaline and digested it partially, and produced on potato a thick, greenish yellow to bright yellow to yellow-brownish slimy growth. The general character of the smallest rods, developed from the dwarfed growth, was sometimes very similar to that of *Bacterium turcosum* (Zimm.) Lehm. et Neum., while the larger motile or immotile yellow rods resembled more those strains described as *Bacterium ochraceum* and *Bacterium fulvum* (Zimm.) Lehm. et Neum.

The white rods observed with *Azotobacter agile* and *A. vitreum* either produced fluorescence or left the color of the substrate unchanged In both cases, however, morphology, flagellation, reproductive organs, colony formation, and growth on the various media were those of a *Bacterium fluorescens* which did not liquefy gelatine or only weakly (*Bacterium*

putidum Lehm. et Neum.). The fluorescence, when it appeared, was mostly weak and apparently was favored by a slightly acid reaction (as is the case with the typical *A. agile*), while ammonia, contrary to the usual behavior of *Bacterium fluorescens*, remained without effect.

The yellow rod developed from the dwarfed growth of *Azotobacter agile* retained most of the cultural characteristics of its origin; merely the cell form was at least temporarily very much like that of *Bacterium putidum*, and there was a clear tendency, especially in broth cultures, to assume endosporulation, which, however, could not be fully stabilized in this case.

Quite different from the white rods of *Azotobacter chroococcum*, as well as of *A. agile*, are the two strains received from Dr. Mulvania (No. 26 and 27). Both produced large amounts of gas and displayed in every respect the cultural characters of Aerobacter (Coli-Aerogenes group). However, their tendency to make large Azotobacterlike cells was very marked, and therefore we did not feel justified to discard them lightly as "contaminations." Undoubtedly they do not belong to the life cycle of *A. chroococcum* or *A. agile;* but, as Mulvania (*36*), too, noticed gas formation in his Azotobacter cultures, there remains the possibility, or even the probability, that another species of Azotobacter exists, which should be more thoroughly studied. The upgrowth from the gonidia to large cells was very similar to that observed with other Azotobacter strains (fig. 10 on Pl. 1). The tendency to assimilate free nitrogen has been ascertained with several members of the Aerobacter group (*24, p. 688*).

6.—SMALL SPORULATING RODS

Aztobacter vitreum did not produce any sporulating rods, but 18 strains were grown from *A. chroococcum*, *A. Beijerinckii*, and *A. agile*. They again proved to be polymorphous, and equally so with both Azotobacter species. Width and length of the rods, as well as the endospore formation, varied considerably, and the cultural features proved to be equally unstable. But these alterations were by no means erratic; in fact, there was a gradual increase in the size of the rods, a progressive change from polar to central sporulation, and a simultaneous passing from weak to vigorous growth. The smallest rods with polar spores were extremely fragile and little inclined to grow on the substrates used. Frequently reinoculation became necessary, and still losses occurred. On the other hand, when left undisturbed on the same substrate they remained alive for long periods. Two of these strains first appeared in old stock cultures (*26*), and several of them survived when kept for five years in mannite solutions. When tested at the end of this period in beef broth and in mannite-nitrate solution those of *A. chroococcum* and *A. Beijerinckii* grew only in the latter, while those of *A. agile* preferred the beef broth.

Morphologically as well as culturally these small, weakly growing, Gram-negative rods with polar spores are practically identical with *Bacillus terminalis* Mig., as described by Lawrence and Ford (*10*). At first it seemed as if those of the chroococcum type could be clearly differentiated from the agile type by the appearance of the spores; the latter showing ridges similar to those of *B. asterosporus* (A. Meyer) Mig. But the cultural character was distinctly different from that of *B. asterosporus* and very similar to that of the other strains. Ford and his collaborators have seen similar ridges occasionally upon the spores of *B. brevis* Mig., *B. fusiformis* A. M. et Gottheil, and of related forms.

and it is also our experience that this feature is rather unstable even in *B. asterosporus*, as is proved by the permanent absence of ridged star spores in the original culture, obtained from the New York Museum of Natural History.

When the strains show a somewhat more vigorous growth and their cells increase in size, the morphological and cultural characters become identical to those of *Bacillus fusiformis* A. M. et Gottheil, according to the descriptions published by Gottheil (*12*) and by Lawrence and Ford (*10*), with the exception that not only globular spores were produced but also many of oval shape. Comparative tests made with a culture from the New York Museum gave identical results. Only on potato agar nearly all spores were globular, and these showed a pronounced inclination to become normal regenerative bodies. Apparently *B. centrosporus* Ford (*17*) should also be classed as a variety of *B. fusiformis*. The cultural marks are very similar, and the particular form of the rods is equally noticeable with otherwise typical *B. fusiformis* growth (fig. 63 on pl. 6).

The next step in the development of the small sporulating rods of *Azotobacter* chroococcum and A. *Beijerinckii* proved to be a change in cell morphology from the slender, frequently pointed forms of *Bacillus fusiformis* to a more compact appearance with rounded or square ends, centrally located spores, and a change in the cultural character to that of *Bacillus pumilus* A. M. et Gottheil. Ford was unable to obtain a typical culture of this strain, and the one we received from Kral's Museum also did not fully agree with Gottheil's description; it displayed in several respects, especially on potato, unmistakably the characters of *Bacillus fusiformis*, that is, while our cultures gaining in size and vigor of growth changed from the type of *Bacillus fusiformis* to that of *Bacillus pumilus*, the weakened stock culture of the original isolation had followed the opposite course. *Bacillus pumilus* in its typical form makes a very characteristic grayish pinkish brownish smooth growth on potato, well known to the senior author from numerous isolations made under his direction by Bierema (*6*) in 1908. Two of these cultures were still at our disposal, as was also one culture of *Bacillus Freudenreichii* (Miquel) Mig., described by the senior author in 1905 (*23, p. 719*) and later recognized as closely related to *Bacillus pumilus* (*6*). The cultural features were still fairly similar to those of the original isolations, but the spore-formation had ceased entirely, and the cells exhibited clearly a mixture of fungoid and small rod-like growth. As was to be expected from these findings further testing confirmed that this fungoid growth of *Bacillus pumilus* is identical with the white-pink Mycobacterium developed from *A. chroococcum* and *A. Beijerinckii* as well as from *Bacterium lactis viscosum*. It was not difficult to change these spore-free strains of *Bacillus pumilus* and *Bacillus Freudenreichii* by continued cultivation in milk into typical strains of *Bacterium lactis viscosum*. On the other hand we have not yet succeeded in reestablishing the endosporulation, which once was very conspicuous with these strains, whose purity and authenticity are beyond doubt. Successive transfers on potato and heating in beef broth up to 85° C. clearly favored the return to a more typical rod form (fig. 58, 54, and 46 on Plates 5 and 4) and also to the production of fairly resistant zygospores and arthrospores. But a complete success has not been reached thus far, and this experience makes it easy to understand why analogous tests with Azotobacter have been and are so often failures.

Still somewhat larger than *Bacillus pumilus* is a sporulating rod which was developed directly from the dwarfed growth (No. 2 grown

repeatedly from No. 1). A very similar strain was received from the New York Museum of Natural History as *Azotobacter chroococcum* (collection No. 522). Another subculture received from the same source under the same number in 1915 had not shown endospore formation but grew very similarly to our dwarfed Azotobacter strains. It died out after a while, and therefore we can not decide whether the sporulating growth later received from New York was a legitimate offspring or a contamination. On account of the fact that the analogous change occurred repeatedly under our eyes, the former possibility seems to us more probable. The New York strain showed all features of *B. circulans* Jordan (*17, p. 519*), and our own culture (No. 2) was fairly similar except that it was more inclined to become a large sporulating rod like those which we still have to discuss.

The small sporulating rods of *Azotobacter agile*, while resembling very closely the smallest type developed from *A. chroococcum* and *A. Beijerinckii*, did not show any tendency to pass over to the *Bacillus pumilus* type, and this is again in good agreement with the recorded absence of the white-pink fungoid growth in the cultures of *A. agile*. In cell form and colony type these sporulating rods first remained very close to those of the small spore-free rods of *A. agile* (that is, *Bacterium putidum*); later however they did not exhibit any sharp difference from the *Bacillus terminalis-fusiformis* type. They, too, could be transformed into large sporulating rods.

7. LARGE SPORULATING RODS

Here again *Azotobacter chroococcum*, *A. Beijerinckii*, and *A. agile* produced very similar growths. The strains derived from the first-named species proved to be identical, morphologically as well as culturally, with *Bacillus petasites* A. M. et Gottheil (*12*), while those obtained from *A. agile* exhibited more the character of *B. silvaticus* A. M. et Neide (*37*), but these differences are rather inconspicuous. An old stock culture of *B. petasites* received from New York was somewhat reduced in size, but otherwise quite typical. Like all strains developed from our Azotobacter cultures, it grew on beef agar either white, yellow, or brown. These three types of growth are not constant on account of the instability of the cells causing the different pigmentation. The typical, very large, broad, granulated rods produce the characteristic yellow growth; the weakly staining oval cysts, which were discussed above, make a whitish grayish layer; and in the brownish material, which also may give a brown color to the agar, sporulating long rods and threads of more or less regular shape are most frequent (fig. 73, 74, 76, and 77 on Plate 7). Temporarily, of course, one or the other mode of growth may predominate, and in short-termed tests such cultures are liable to be erroneously classified as different "species." Form, color, and structure of the colonies, although very characteristic in their typical development, may vary considerably, too. Occasionally colonies were seen which macroscopically as well as microscopically (low magnification) were indistinguishable from those of the large nonsporulating cells of *A. chroococcum;* they contained nothing but spores.

Illustrated descriptions, of two large, sporulating, nitrogen-fixing organisms (*Bacillus malabarensis*, and *B. danicus*), which were published by the senior author some years ago (*27, 29, 30*) can now be accepted as descriptions of the large sporulating cell type of Azotobacter. A renewed thorough study of our stock cultures left no doubt that they may

assume the character of this organism, as was already indicated in those earlier papers. With regard to *B. danicus* it was said in 1908 that all cell forms characteristic of Azotobacter were seen except the sarcina form; with the help of our newly won knowledge this could now be easily developed from secondary colonies on beef agar. The curious beet-shaped large cells of *B. malabarensis* can now also be identified with the gonidangia of the sporulating Azotobacter strains, and that the original isolation produced in mannite solution large globular spore-free Azotobacter-like cells (*27*) is also easily understood at present.

Bacillus luteus Baker et Smith seems to be identical with *B. petasites*, according to the detailed description published by Garbowski (*11*). The three types of growth (white, yellow, or brown), the inclination to produce in secondary colonies yellow spore-free cells (coccoids), and the various cultural marks are the same in both cases. The ability to fix free nitrogen is, of course, variable; *B. malabarensis* and *B. danicus* were found to assimilate 1 to 2 mgm. per 100 cc. 1 per cent mannite soil extract.

The potato cultures, which proved most helpful for reestablishing the spore-free typical Azotobacter growth, gave also the only opportunity to distinguish with some accuracy the sporulating strains developed from *Azatobacter chroococcum* and *A. Beijerinckii* and those of *A. agile*. For one or two weeks all of them displayed first the whitish slimy, later the yellow glossy growth characteristic of *Bacillus petasites;* but then the growth became distinctly brown when derived from *A. chroococcum* or *A. Beijerinckii*, while it turned gray with *A. agile* as with *Bacillus silvaticus*. However, a much better differentiation was secured if use was made of the conspicuous inclination of these potato cultures to give rise to other types of growth. The cultures developed from *A. chroococcum* and *A. Beijerinckii* were found inclined to resume the character of *B. pumilus*, or transferred to beef gelatine they even went back to the very characteristic white-pink fungoid growth, while those of *A. agile* returned on beef gelatine to the white, small nonsporulating nonliquefying rod closely related to *Bacterium putidum*. Still more convincing, although more difficult to attain, is of course the reestablishment of the typical nonsporulating large cells; continued culture on potato alternated with transplants to alkaline mannite-nitrate agar and solution gave comparatively the most satisfactory results in this respect.

That the large sporulating rods of the Megaterium type are much inclined to become smaller and to assume other cultural characters was often observed, and it is equally well known that stock cultures of the smaller sporulating rods not infrequently lose their ability to produce endospores and become similar to *Bacterium coli* and related forms. With the anaerobic nitrogen-fixing bacilli of the Amylobacter group these changes are even more frequent, and the ability of these organisms to produce aerobic nonsporulating coccoids brings them into closer contact with the aerobic nitrogen-fixing bacilli of the Azotobacter group. All these relations should be properly considered and more thoroughly studied.

DISCUSSION OF RESULTS

The observations recorded concerning the different types of vegetative growth and of reproduction of Azotobacter permit a much more complete and much more accurate characterization of this organism than has been given thus far to any other genus of bacteria. In fact, all form genera of

bacteria, as created by Ferdinand Cohn and his successors, have become invalid because, in accordance with earlier observations discussed in Part I (*25*), our experiments have shown that Azotobacter, like certain other natural groups of bacteria, is able to grow in all forms which were accepted as a basis for establishing the so-called genera Micrococcus, Bacterium, Pseudomonas, Bacillus, and Mycobacterium. Contrary to a very widespread, although quite illogical, assumption the pleomorphism of Azotobacter, as of all other bacteria, does not obscure but clarifies and strengthens the character of the genus as well as of its species. While a study of the large nonsporulating cells alone made it probable that *Azotobacter Beijerinckii* J. G. Lipman might be a variety of *A. chroococcum* Beij., this conclusion has been made certain by the discovery that all other types of growth are identical in both cases. *A. agile*, on the other hand, exhibits in its large nonsporulating cell form certain features which might be accepted as proof that it should be classed as another variety of *A. chroococcum*, as was done, for example, by Prazmowski (*40*); yet its small nonsporulating rod form and its fungoid growth are so different that no doubt remains about its being a true species. That *A. Vinelandii* J. G. Lipman is identical with *A. agile* Beij. could be said on account of the identity of their large nonsporulating cell forms (*30*), and all other developmental stages have now shown themselves equally identical. *A. vitreum* Löhnis, in its large nonsporulating cell type apparently quite different from the other species, has displayed in all other directions so much similarity with *A. agile* that its being a variety of this species is practically certain, although this conclusion had to be drawn from the results obtained with the only strain available.

Three other Azotobacter species have been described as *Azotobacter Woodstownii* J. G. Lipman (*22*), *A. Hilgardi* C. B. Lipman (*19*), and *A. Smyrnii* C. B. Lipman et Burgess (*20*); but they all should be canceled. The first one was never shown to be able to fix nitrogen, and its characterization is not distinct enough. The second one was only very incompletely described. According to information kindly furnished by its author it was "very similar" to *A. Smyrnii*. This like the other is no longer alive, but its illustrated description (*20*) leaves no doubt that it was identical with the sporulating large cell form of *A. chroococcum*. It is true that the original description says motility and spores were lacking; but the characterization of what was accepted as vegetative cells makes it certain that only spores were seen and photographed. Every mark ascribed to *A. Smyrnii* tallies exactly with those of quickly sporulating large cells of *A. chroococcum* (Bacillus petasites).

The ability of Azotobacter to produce in certain stages of growth genuine heat-resistant endospores was accepted by the senior author (*26*) as proof that it should be classed as *Bacillus azotobacter* among the sporulating bacilli. But after our more recent studies have shown conclusively that all old form genera, including that of *Bacillus* F. Cohn emend. Hueppe, will have to be replaced gradually by natural genera, based on complete investigation of their life histories, it appeared preferable to retain and to emend the genus *Azotobacter* Beij. by adding to the large nonsporulating type of growth the six other growth types described on the preceding pages.

Contrary to our observations upon endosporulation in Azotobacter cells it was asserted by D. H. Jones (*16*), as well as by the Committee of the Society of American Bacteriologists on Characterization and Classifi-

cation of Bacterial Types (*45*) that Azotobacter does not produce heat-resistant endospores. As an analogous assertion had been made by this Committee in a preliminary report, the senior author pointed out at the Annual Meeting of the Society in 1917 that this opinion was contrary to the facts recorded by him in 1914 and 1916 (*26*, *28*). The final report of the committee, however, still retains the statement that Azotobacter does not produce heat-resistant spores. Jones' assertion is in conflict with our observations and, furthermore, seems to be contradicted by his own data. He reports (*16*, *p. 328*) that some of his stock cultures—

> produced colonies of encapsulated spore-forming rods both large and small.

Because these sporulating rods did not grow like Azotobacter, he concluded that they were contaminations. That this assumption may have been correct is of course possible, but it is equally possible that the facts observed are further proof that endosporulating rods may be produced by Azotobacter. We tried to make it clear in our first paper on this subject (*26*) that naturally the sporulating cells of Azotobacter differ in their growth as in their appearance more or less from the nonsporulating cells, but that the possibility to change them back to the original nonsporulating form proves beyond doubt that they are really a type of growth of Azotobacter and no "contamination." To assume that morphologically different cells should display the same physiological (cultural) character is, of course, quite illogical. This point was emphasized in the following statement in our preliminary paper (*28*, *p.690*):

> Such wide morphological differences must always be connected with no less considerable alterations of the whole physiological character, so that these other types, if they are known, of course, are stored away as entirely different "species" in various remote places in the so-called "system" of bacteria. This conclusion can be drawn with absolute certainty from our observations on *B. azotobacter* as well as from Henri's experiments with *B. anthracis*. If only those changed forms, frequently seen in all bacteriological laboratories, had not been persistently discarded as uninteresting "involution forms" or as "contaminations," the whole situation would undoubtedly be much clearer and much more satisfactory.

Transformations from and to the original type of growth alone are decisive. That they can not be expected in short-termed experiments conducted along the customary lines of bacteriological research is amply proved by the facts recorded in this and in the first part of our Studies upon the Life Cycles of the Bacteria (*25*). Negative results based upon such experiments are not conclusive.

With reference to the question of conjunction of bacterial cells, Jones (*17*, *p. 330*) states—

> that what is here referred to as conjunction of two individual cells is rather the incomplete fission of individual cells in process of division.

That there were "side connections" which drew our attention to this point, and that these lateral bridges are clearly visible in our photographs can not be explained, however, upon the assumption of incomplete fission. That also in this case we merely rediscovered a fact which should long have found its place in the bacteriological text books was shown beyond doubt by the data we were able to collect and to present in Part I (*25*, *p. 197–204*).

That pathogenic bacteria—for example, the causative agents of bubonic plague, anthrax, cholera, diphtheria, etc.—are just as pleomorphous as Azotobacter, Nitrosomonas, *Bacterium radicicola*, etc., and that their

life histories follow the same fundamental lines can no longer be denied, provided that the facts recorded in the literature are taken under adequate consideration. In morphological, as well as in physiological respects, much interesting information is to be expected from such investigations.

As was frequently noticed in bacteriological tests of soils, wherein usually a normal growth of Azotobacter is to be observed, this organism may be temporarily replaced by other nitrogen-fixing bacteria, such as *Bacterium lactis viscosum* and other nonsporulating rods, various large sporulating bacilli, some micrococci, etc. The possibility that different developmental stages of the same organism may have to be made responsible for such changes sheds new light upon the very great ability of the bacteria to adapt themselves to widely varying environmental conditions. It is especially noteworthy that the life cycle of Azotobacter not only unites several of the best-known aerobic nitrogen- and nitrate-assimilating organisms, but also strong ammonia producers and forms which are known to be very able to make use of the various organic substances of the soil, first of all of the humates, namely, several mycobacteria (*41*, *43*).

SUMMARY

Tests made with 30 strains of Azotobacter and with several cultures of related bacteria have shown that only two species of Azotobacter are completely characterized thus far: *Azotobacter chroococcum* and *A. agile* Beij. (syn. *A. Vinelandii* J. G. Lipman). *A. Beijerinckii* J. G. Lipman is a variety of *A. chroococcum* and *A. vitreum* Löhnis is probably a variety of *A. agile*. *A. Smyrnii* C .B. Lipman et Burgess can not be accepted as a species; according to all marks ascribed to it by its authors it is the large sporulating growth type of *A. chroococcum*. *A. Hilgardi* C. B. Lipman and *A. Woodstownii* J. G. Lipman are both incompletely described and should not be retained.

The genus Azotobacter is characterized by the morphological and physiological behavior of its seven different cell types. These are (1) large nonsporulating globular, oval, or rodlike cells of white, yellowish, or brown color, with polar or peritrichous flagella, able to act as gonidangia and microcysts; (2) coccoid cells of white, yellow, or pink pigmentation, the vegetative growth of the regenerative bodies, identical with *Micrococcus concentricus* Zimm., *Micrococcus sulfureus* Zimm. emend. Lehm. et Neum., and with *Micrococcus roseus* (Bumm) Lehm et Neum., respectively; (3) dwarfed cell type of yellow, white, or of red color, the vegetative growth of the gonidia; (4) irregular, fungoid cells, producing a yellow, orange, or (in the case of *A. chroococcum* and *A. Beijerinckii*) a white or pink growth; the former two are closely related to the dwarfed growth and identical with *Mycobacterium luteum* Söhngen and with *Mycobacterium lacticola* Lehm. et Neum., respectively, while the latter is probably identical with *Mycobacterium album* Söhngen; (5) small nonsporulating rods of white or of yellow color, the former being identical with *Bacterium lactis viscosum* (Adametz) Lehm. et Neum. in the case of *A. chroococcum* and *A. Beijerinckii*, but with *Bacterium putidum* (Flügge) Lehm. et Neum. in the case of *A. agile* and *A. vitreum;* (6) small sporulating rods, identical with *Bacillus terminalis* Mig., *Bacillus fusiformis* A. M. et Gottheil, and in the case of *A. chroococcum* and *A. Beijerinckii* with *Bacillus pumilus* A. M. et Gottheil; (7) large sporulating cells, growing white, yellow, and brown, identical with *Bacillus luteus* Baker et Smith, *Bacillus petasites* A. M. et Gottheil, *Bacillus malabarensis*

Löhnis et Pillai and *Bacillus danicus* Löhnis et Westermann; those grown from *A. agile* showed some marks of *Bacillus silvaticus* A. M. et Neide, which, however, is closely related to *Bacillus luteus*. All cell types were transformed into each other.

The reproductive organs of Azotobacter are (1) gonidia, in part filterable, produced in small numbers, one to four, in the small cells, and in larger quantities in the large ones, which act in this case as gonidangia; (2) regenerative bodies and exospores, either produced by the cells in lateral or in terminal position, or growing up from the symplasm; (3) arthrospores, formed by fragmentation of the rod-like or fungoid cells; (4) microcysts, that is, small or large globular or oval resting cells; (5) endospores, produced singly by the rod-like cells in terminal or in central position, or to two or more in globular or spindle-shaped sporangia. Gonidia form the basis for the development of regenerative bodies, arthrospores and endospores; the production of polar regenerative bodies always preceded the establishment of endosporulation, and endospores were found to be able to reproduce gonidia and regenerative bodies.

The formation of the symplasm and the regeneration of new cells from this more or less amorphous substance of varying stainability proceeds with Azotobacter in the same manner as with all other bacteria. Amoeboid movement of symplasm was never observed, but strong inner movements were seen, and occasionally globular macrocysts were found which are analogous to the macroplasts discovered by Lankester. Besides normal cells, relatively solid agglomerations of more or less irregular shape were produced by the symplasm, so-called sclerotia, which later either transformed themselves to vegetative or reproductive cells or reentered the symplastic stage. Large "filidia," representing another type of more or less irregular and ephemeric upgrowth from the symplasm, were also observed.

Conjunction was regularly seen in young cultures before the formation of gonidia, regenerative bodies, and of exospores and endospores took place. Part of the regenerative bodies are clearly zygospores. The cell union is either temporary, effected by connecting beaks, bridges, or by direct contact of two or more cells, or permanent due either to a sticking together of two uniform cells, which retain their identity, or to a coalescence of two cells of more or less different appearance. These various modes of conjunction, observed with Azotobacter as with other bacteria, resemble very closely those recorded with yeasts and with protozoa.

The fact that the different developmental stages of Azotobacter could be in part identified with certain so-called species belonging to the form genera Micrococcus, Bacterium, Pseudomonas, Bacillus, and Mycobacterium demonstrates anew and conclusively that the whole system of bacteria needs complete revision, which is to be based upon the results of thorough examination of the life histories of the bacteria.

Some bacteriologists will be inclined to explain the phenomena reported in this paper by assuming accidental contaminations of the cultures studied. However, careful consideration of the data reported will show that most of the changes shown can not reasonably be explained upon the hypothesis of contaminations and that there are no changes reported where the reasons that might be imagined for the adoption of the contamination hypothesis deserve any more consideration than the reasons advanced for considering them to be normal changes in the life cycle of Azotobacter.

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NOTE.—All preparates (Plates 1-9) were stained with aqueous fuchin. Magnification in all cases X900.

PLATE 1

Large non-sporulating cells.

Fig. 1.—Large cells made up of nuclear material and slime. *Azotobacter chroococcum* (No. 24). Mannite soil extract, 2 days.

Fig. 2.—Large globular, oval, and rod-like cells, cells of medium size with nuclei. *A. chroococcum* (No. 14). Mannite-nitrate solution, 5 days.

Fig. 3.—Nuclei of cells of medium size liberated making small cells. *A. agile* (No. 16). Mannite-nitrate agar, 6 weeks.

Fig. 4.—Liberated nuclear material making regenerative bodies. *A. chroococcum* (No. 22). Mannite-nitrate solution, 7 days.

Fig. 5.—Large cells producing gonidia and regenerative bodies. *A. chroococcum* (No. 12). Beef agar, 3 weeks.

Fig. 6.—Regenerative bodies produced by large cells. *A. chroococcum* (No. 18). Beef agar, 5 days.

Fig. 7.—Large round and rod-like cells. *A. agile* (No. 16c). Beef agar, 2 days.

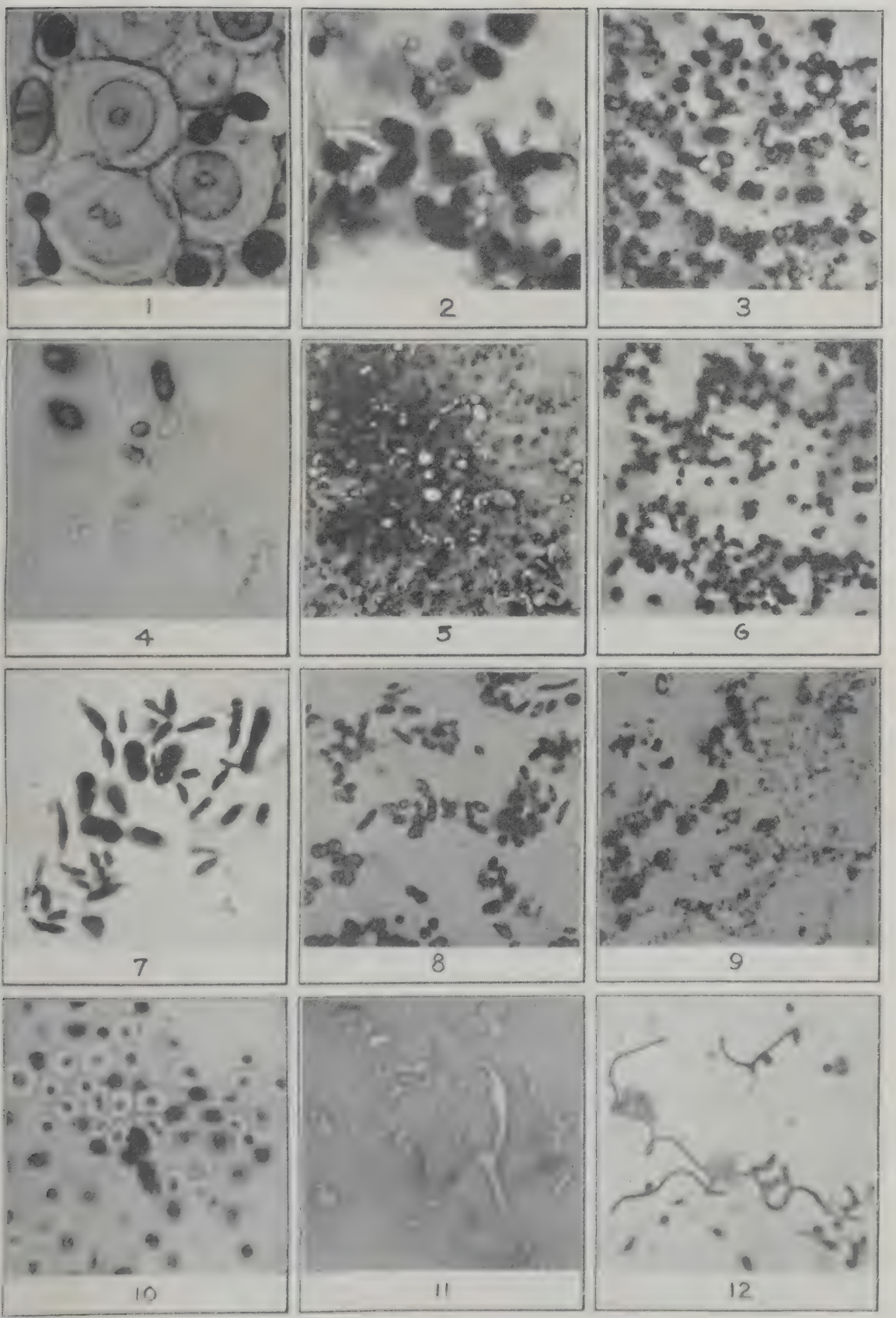
Fig. 8.—Typical mixture of large and medium-sized cells. *A. chroococcum* (No. 11). Mannite-nitrate agar, 1 week.

Fig. 9.—Typical mixture of medium-sized and small cells in old culture. *A. agile* (No. 16). Mannite-nitrate agar, 11 months.

Fig. 10.—Upgrowth from small to large cells. *A.* sp. (No. 26). Mannite-nitrate solution, 5 days.

Fig. 11.—Liberation of gonidia from regular and irregular cells. *A. chroococcum* (No. 22). Mannite-nitrate solution, 5 weeks.

Fig. 12.—Spirochaetoid forms, branched and budding cells. *A. Beijerinckii* (No. 13). Beef agar, 1½ month.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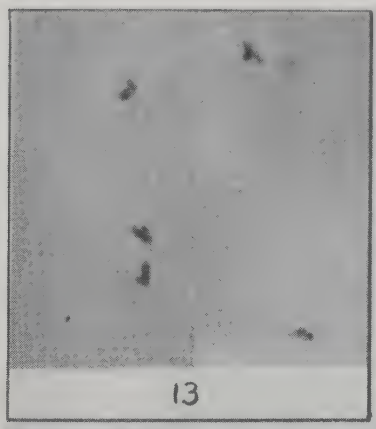
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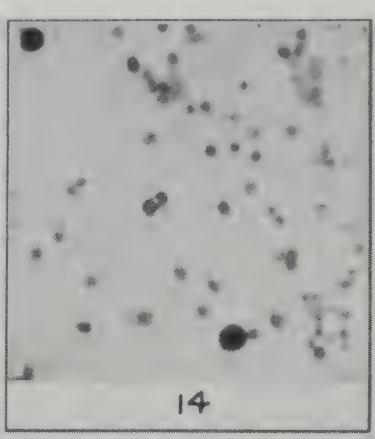
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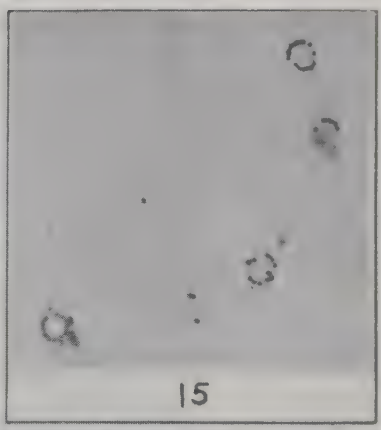
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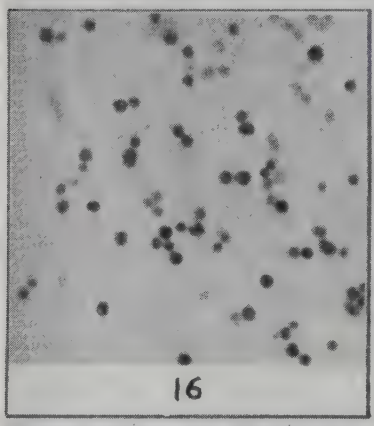
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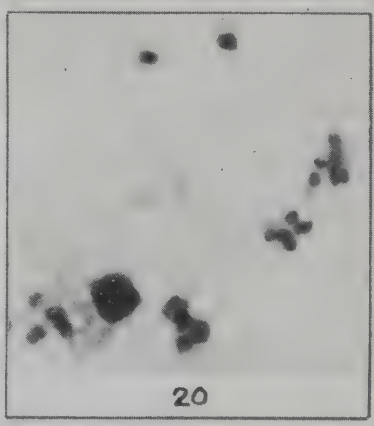
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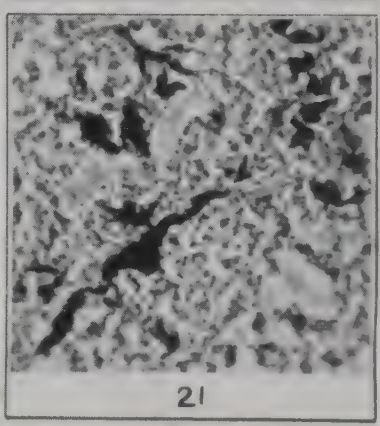
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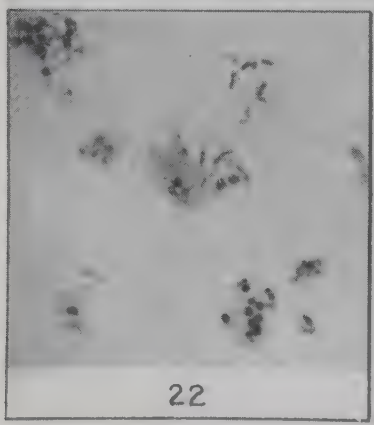
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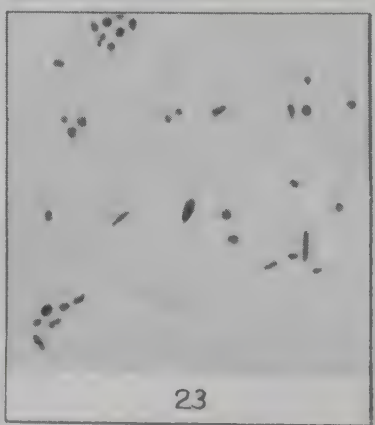
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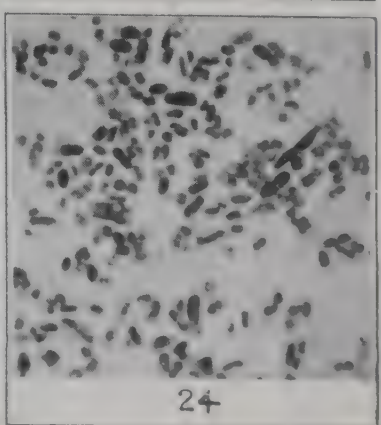
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PLATE 2

Coccoid cells.

Fig. 13.—Gonidia budding. *Azotobacter chroococcum* (No. 24). Mannite-nitrate solution, 1 day.

Fig. 14.—Gonidia growing to regenerative bodies. *A. chroococcum* (No. 18). Potato agar, 1 week.

Fig. 15.—Small regenerative bodies growing from large pale cells. *A. chroococcum* (No. 10). Mannite-nitrate agar, 6 days.

Fig. 16.—Regenerative bodies multiplying by budding and fission. *A. chroococcum* (No. 25). Mannite-nitrate solution, 2 days.

Fig. 17.—Typical coccoid growth. *A. Beijerinckii* (No. 6). Potato agar, 3 weeks.

Fig. 18.—Coccoid cells of small size. *A. chroococcum* (No. 10). Mannite-nitrate agar, 4 weeks.

Fig. 19.—Upgrowth from small to large coccoid cells. *A. chroococcum* (No. 19). Beef agar, 10 days.

Fig. 20.—Regeneration of large cells. *A. agile* (No. 7). Milk, 4 weeks.

Fig. 21.—Coccoids and sclerotia from symplasm. *Bacillus Freudenreichii* (No. 62). Beef agar, 1 week.

Fig. 22.—Coccoids reproducing small rods. *A. Beijerinckii* (No. 15). Potato agar, 5 weeks.

Fig. 23.—Coccoids reproducing small sporulating rods. *A. chroococcum* (No. 19). Mannite-nitrate solution, 5 days.

Fig. 24.—Coccoids reproducing cells of different size and shape. *A. chroococcum* (No. 25). Potato, 4 days.

PLATE 3

Dwarfed growth.

Fig. 25.—Typical gonidial growth. *Azotobacter Beijerinckei* (No. 6). Beef agar, 3 weeks.

Fig. 26.—Typical gonidial growth. *A. chroococcum* (No. 17). Mannite-nitrate agar, 5 days.

Fig. 27.—Dwarfed cells producing regenerative bodies. *A. chroococcum* (No. 1). Mannite-nitrate agar, 2 weeks.

Fig. 28.—Gonidia and slime, beginning formation of fungoid cells. *A. vitreum* (No. 9). Mannite-nitrate agar, 5 days.

Fig. 29.—Formation of fungoid cells from gonidia. *A. vitreum* (No. 9). Mannite-nitrate agar, 7 days.

Fig. 30.—Dwarfed cells assuming fungoid growth. *A. chroococcum* (No. 17). Beef agar, acid, 16 days.

Fig. 31.—Gonidia growing to small non-sporulating rods. *A. chroococcum* (No. 1). Potato agar, 7 weeks.

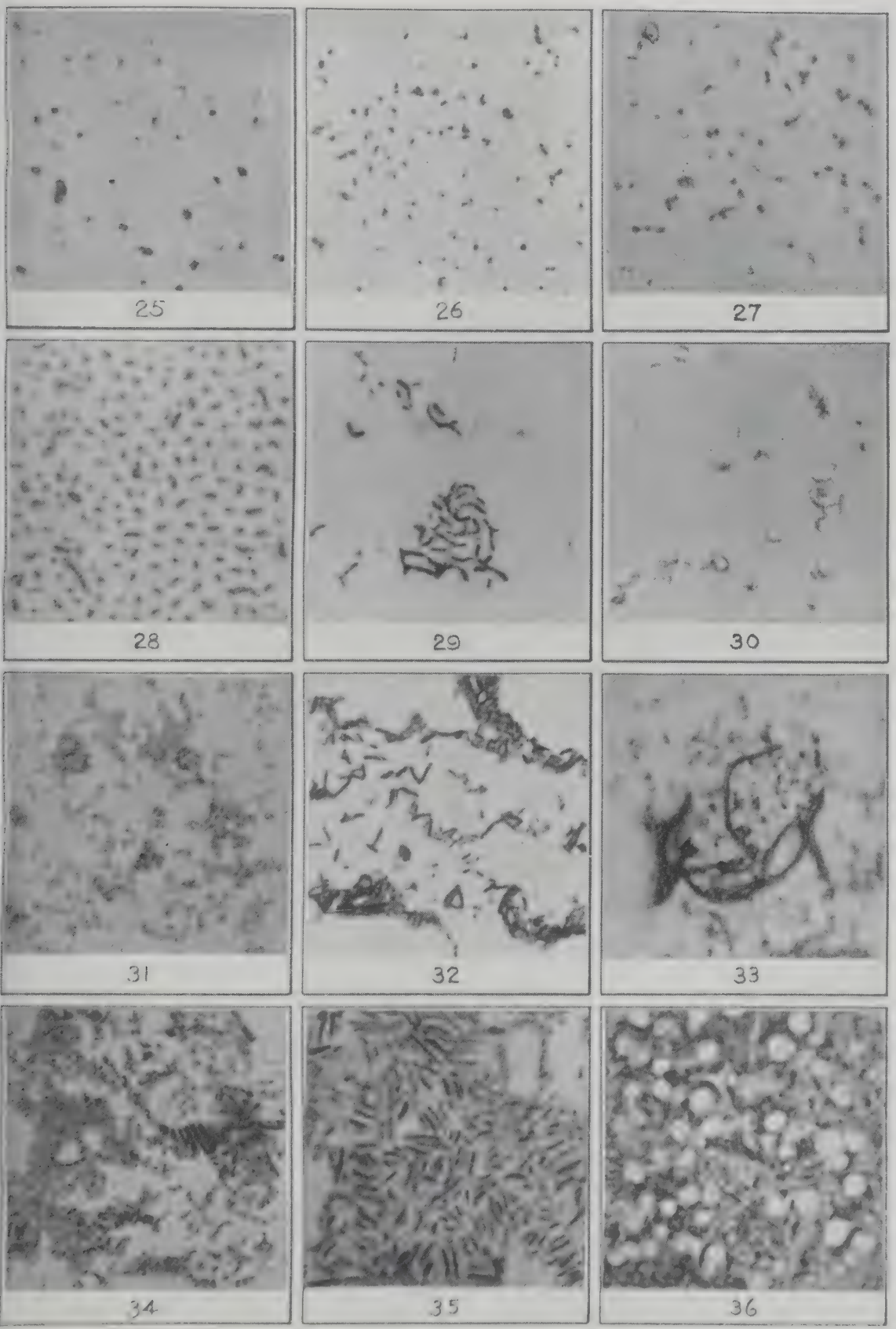
Fig. 32.—Gonidia growing to small and large rods. *A. chroococcum* (No. 1). Beef agar, 11 days.

Fig. 33.—Symplasm reproducing dwarfed growth and large slime threads (filidia). *A. chroococcum* (No. 1). Potato agar, 3 weeks.

Fig. 34.—Dwarfed growth and rods of different size from symplasm. *A. chroococcum* (No. 1). Mannite-nitrate agar, 6 days.

Fig. 35.—Upgrowth of large rods and globules from dwarfed growth. *A. chroococcum* (No. 1). Beef agar, 11 days.

Fig. 36.—Development of dwarfed cells, large globules, and branched filidia from symplasm. *A. chroococcum* (No. 1). Potato agar, 3 weeks.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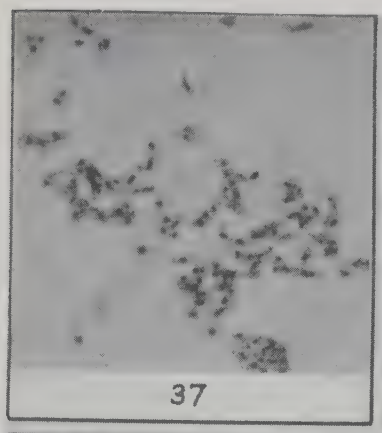

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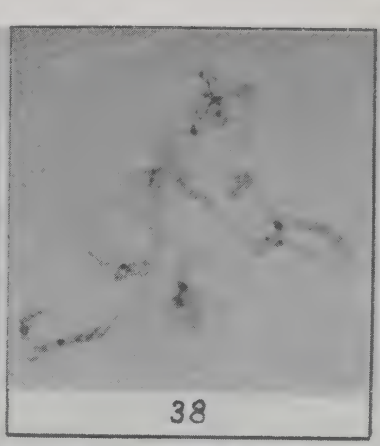

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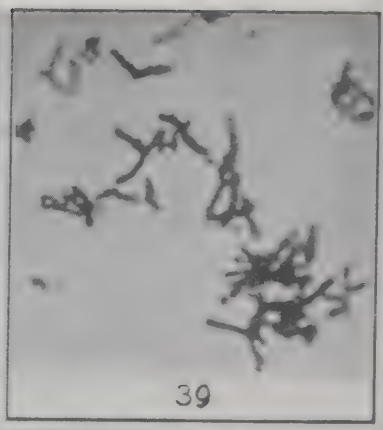

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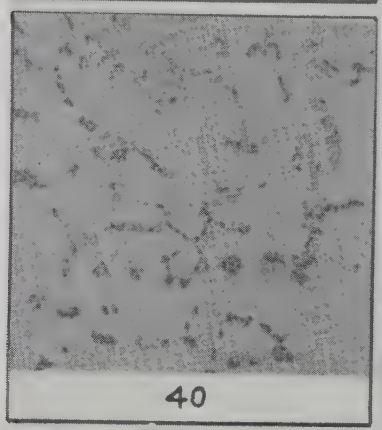

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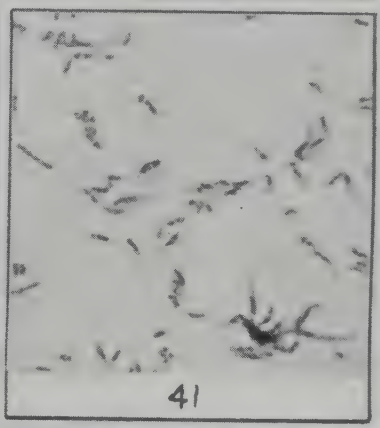

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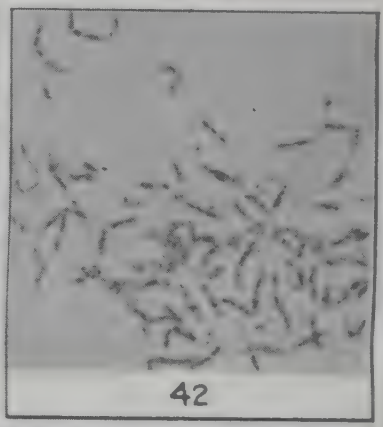

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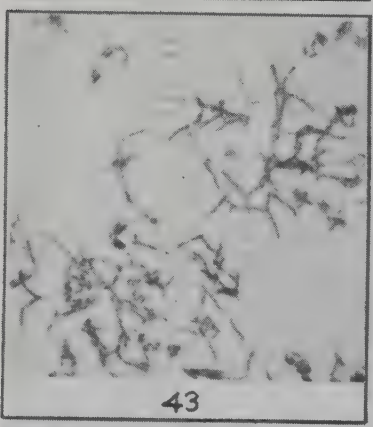

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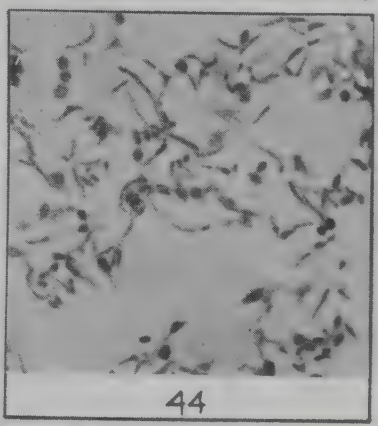

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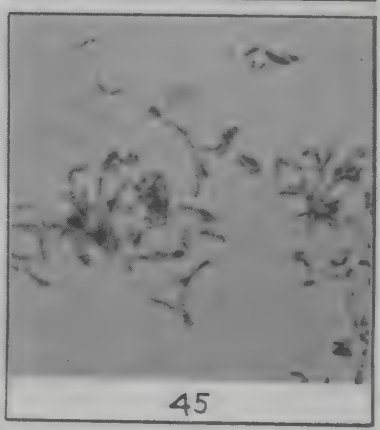

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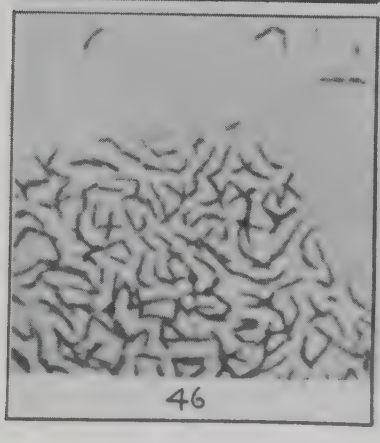

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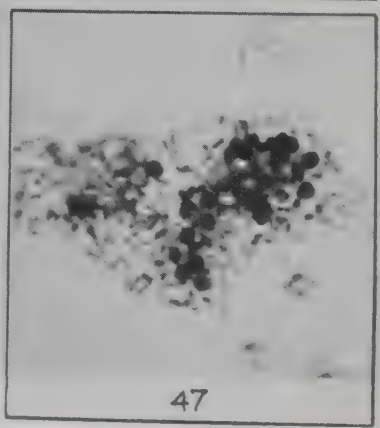

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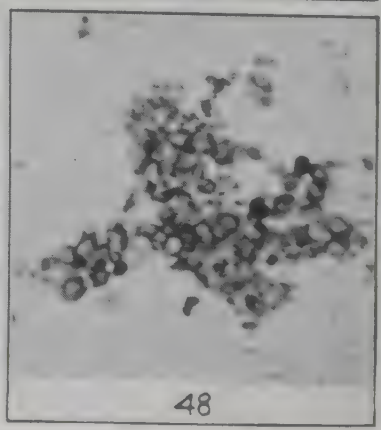

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PLATE 4

Fungoid cell type.

Fig. 37.—Typical fungoid growth. *A. Beijerinckii* (No. 15). Mannite-nitrate agar, 5 days.

Fig. 38.—Large fungoid growth. *A. Beijerinckii* (No. 15). Mannite-nitrate agar, 5 days.

Fig. 39.—Large fungoid growth, well stained. *A. chroococcum* (No. 14). Potato, 1 week.

Fig. 40.—Slime threads liberating gonidia. *A. chroococcum* (No. 17). Mannite-nitrate agar, 5 days.

Fig. 41.—Slime threads producing gonidia and small rods. *Bacterium lactis viscosum* (No. 90). Mannite-nitrate agar, 2 days.

Fig. 42.—From fungoid to coccoid and rod forms. *Bacterium lactis viscosum* (No. 90). Potato, 4 weeks.

Fig. 43.—From fungoid growth to small spore-free rods. *A. Beijerinckii* (No. 15). Beef broth, 3 weeks.

Fig. 44.—From fungoid to coccoid and sporulating growth. *A. Beijerinckii* (No. 15). Potato agar, 12 days.

Fig. 45.—From fungoid growth to spore-free and sporulating rod forms. *A. Beijerinckii* (No. 15). Beef broth, 4 weeks.

Fig. 46.—From fungoid growth to sporulating rods. *Bacillus pumilus* (No. 61). Beef broth, heated to 75° C., 1 week.

Fig. 47.—Fungoid, rod-like, and large globular cells from symplasm. *A. Beijerinckii* (No. 15). Mannite-nitrate solution, 2 weeks.

Fig. 48.—Coccoid, fungoid, and large globular cells from symplasm. *Bacterium lactis viscosum* (No. 89b). Beef agar, 1 week.

PLATE 5

Small non-sporulating rods.

Fig. 49.—Coccobacilli derived from large cells. *Azotobacter Beijerinckii* (No. 15). Mannite-nitrate agar, 7 days.

Fig. 50.—Typical coccobacilli. *A. Beijerinckii* (No. 15). Beef agar, 7 days.

Fig. 51.—Small rods with gonidia and regenerative bodies. *A. chroococcum* (No. 25). Potato, 3 weeks.

Fig. 52.—Tendency to make longer sporulating rods. *A. Beijerinckii* (No. 15). Potato, 6 days.

Fig. 53.—From small rods to fungoid and sporulating cells. *A. Beijerinckii* (No. 15). Beef broth, 4 weeks.

Fig. 54.—Tendency to return to sporulation. *Bacillus pumilus* (No. 60). Beef gelatine, 7 days.

Fig. 55.—From small rods to fungoid and globular growth. *A. vitreum* (No. 19). Salt agar, 3 days.

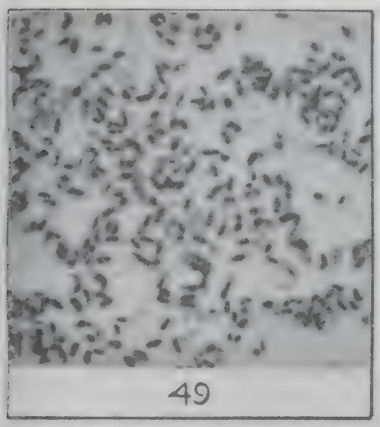
Fig. 56.—Small rods and large globules growing from symplasm. *A. agile* (No. 7c). Beef agar, 3 weeks.

Fig. 57.—Small rods producing Azotobacter-like gonidangia. *Bacterium lactis viscosum* (No. 89b). Mannite-nitrate agar, 1 week.

Fig. 58.—Small rods assuming fungoid growth. *Bacillus pumilus* (No. 61). Milk, 2 weeks.

Fig. 59.—Small and large rods growing from symplasm. *A. agile* (No. 7c). Potato agar, 1 week.

Fig. 60.—Small and large rod-like and round cells growing from symplasm. *A. agile* (No. 16b). Beef agar, 1 week.



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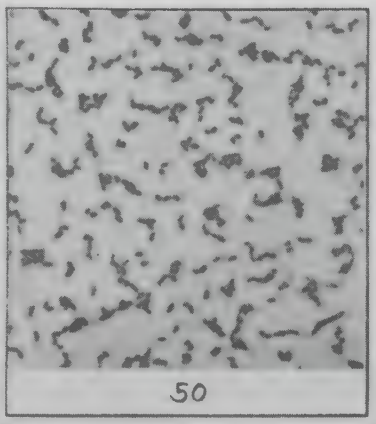

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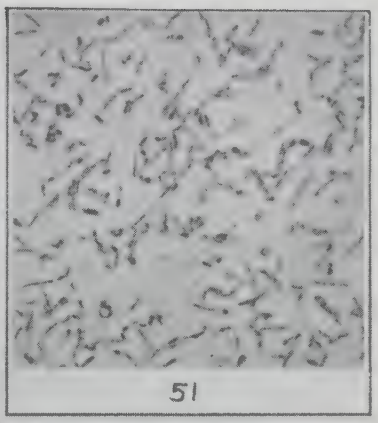

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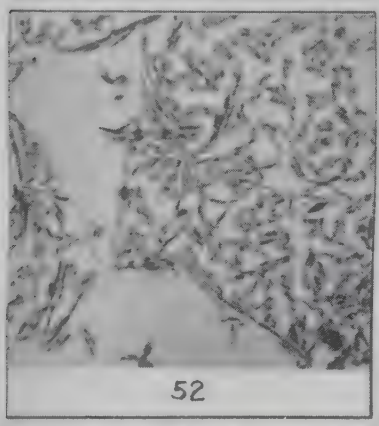

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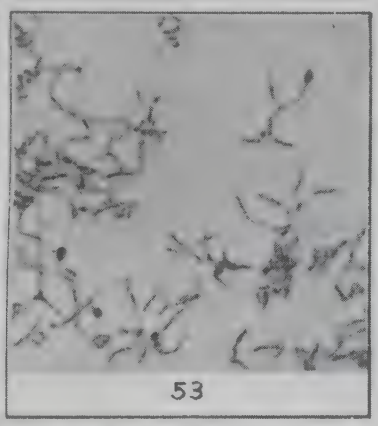

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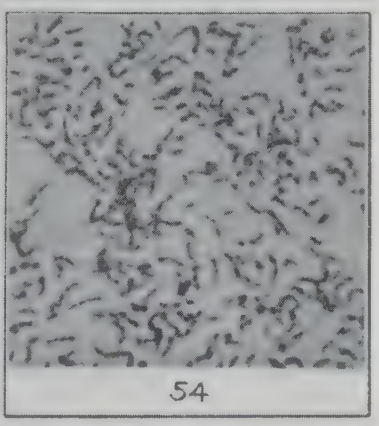

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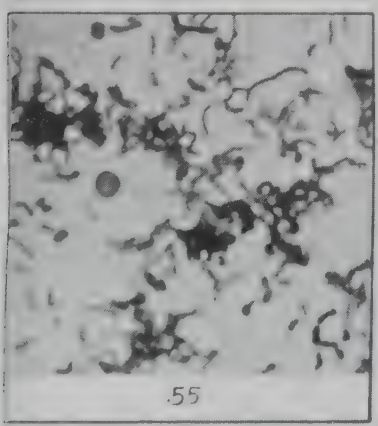

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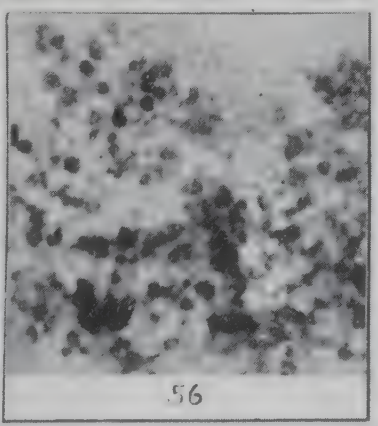

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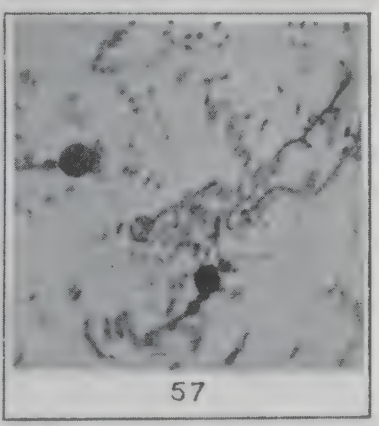

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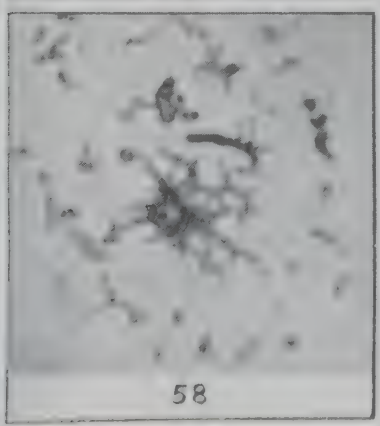

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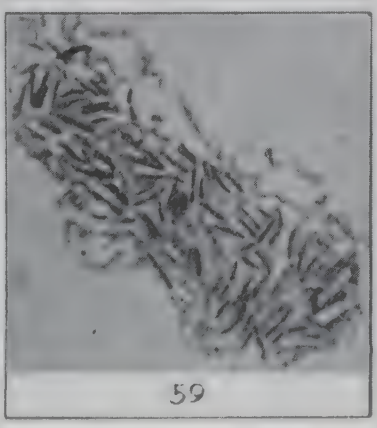

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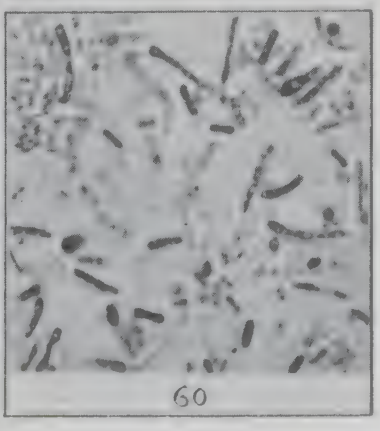

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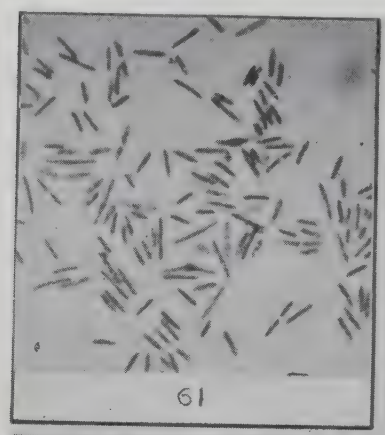
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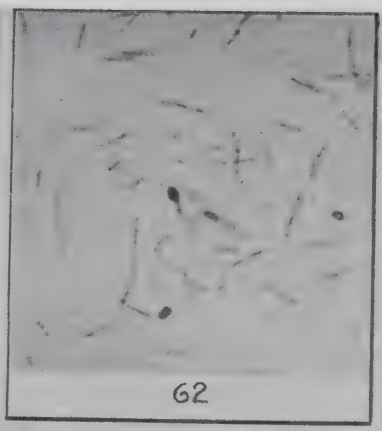
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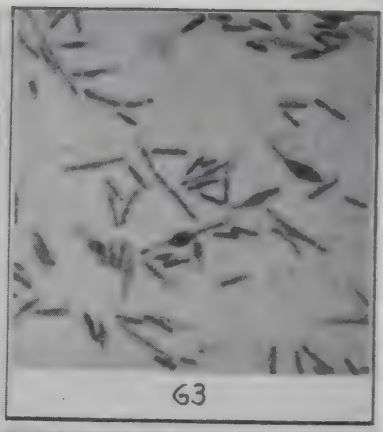
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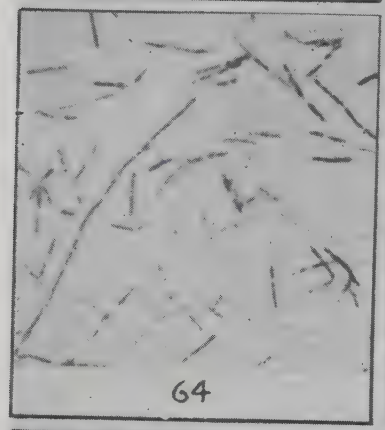
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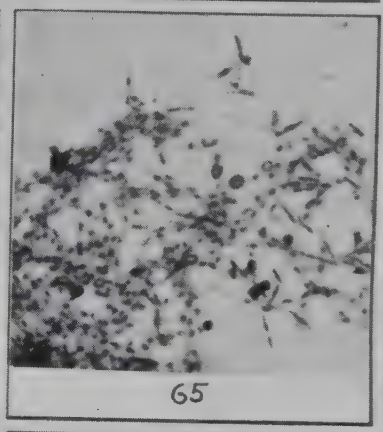
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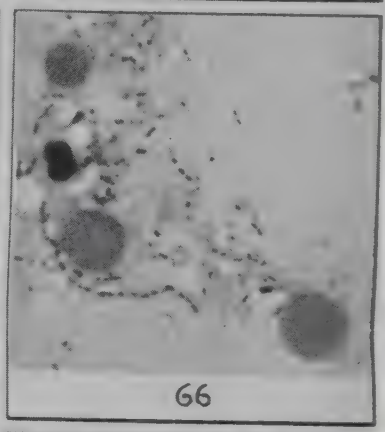
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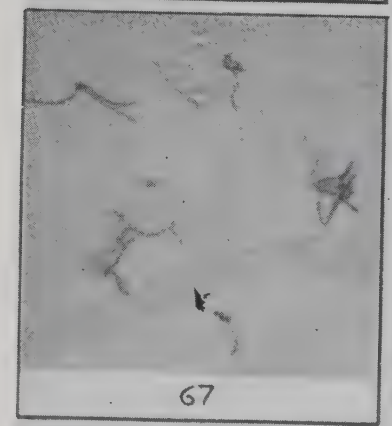
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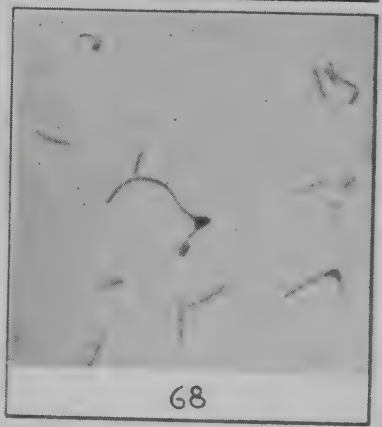
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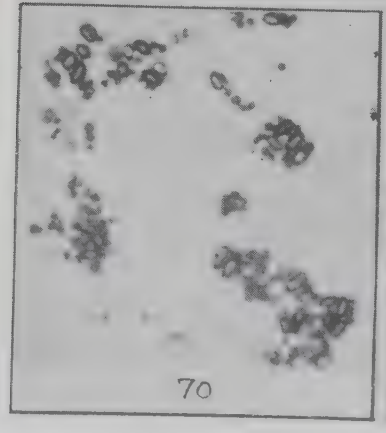
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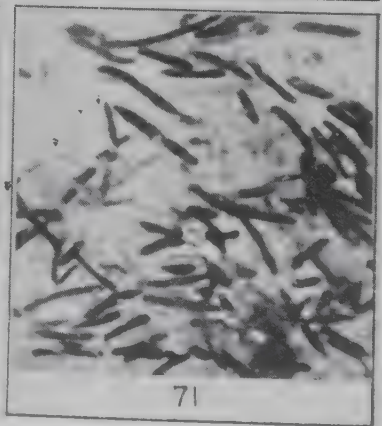
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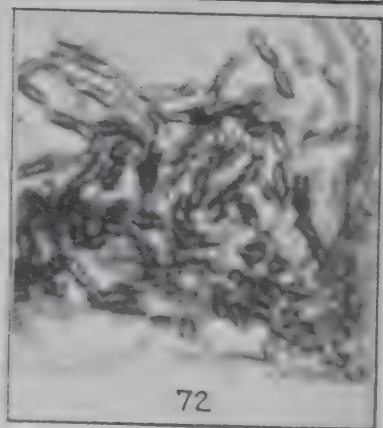
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PLATE 6

Small sporulating rods.

Fig. 61.—Typical sporulating rods. *Azotobacter Beijerinckii* (No. 5). Beef agar, 2 days.

Fig. 62.—Weakened sporulating rods. *A. agile* (No. 7). Mannite-nitrate solution, 3 weeks.

Fig. 63.—Larger sporulating rods. *A. chroococcum* (No. 19). Beef agar, 10 days.

Fig. 64.—Threads producing gonidia. *A. Beijerinckii* (No. 5). Milk, 2 months.

Fig. 65.—Threads producing gonidia, regenerative bodies, and endospores. *A. Beijerinckii* (No. 6). Beef broth, 1 week.

Fig. 66.—Arthrospore formation; encysted symplasm. *A. Beijerinckii* (No. 6). Milk, 7 weeks.

Fig. 67.—Rods producing gonidia and regenerative bodies. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 68.—Conjunction and zygospore formation. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 69.—Formation of gonidangia and regenerative bodies. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, 4 weeks.

Fig. 70.—Spores producing regenerative bodies. *A. Beijerinckii* (No. 6). Beef agar, 2 weeks.

Fig. 71.—Upgrowth to large sporulating rods. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, P_H 8.0, 3 weeks.

Fig. 72.—Development to large sporulating rods. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, P_H 7.5, 4 weeks.

PLATE 7

Large sporulating cells.

Fig. 73.—Typical large sporulating cells. *Azotobacter Beijerinckii* (No. 3). Potato, 2 weeks.

Fig. 74.—Microcysts and vegetative rods.—*A. chroococcum* (No. 25). Beef agar, 1 week.

Fig. 75.—Branched rods and threads. *A. Beijerinckii* (No. 3). Beef agar, 12 days.

Fig. 76.—Threads with different segments. *A. Beijerinckii* (No. 4). Beef agar, 2 weeks.

Fig. 77.—Threads with gonidia and regenerative bodies. *A. Beijerinckii* (No. 3). Beef agar, 5 days.

Fig. 78.—Thread segmenting to globular cells. *A. chroococcum* (No. 18). Mannite soil extract, 3 weeks.

Fig. 79.—Globular sporangia producing 1 to 3 spores. *Bacillus danicus* (No. 34). Mannite-nitrate agar, 2 weeks.

Fig. 80.—Globular cells dividing to 2 curved rods. *A. chroococcum* (No. 20). Potato, 1 week.

Fig. 81.—Branched sporangia. *A. chrococcum* (No. 25). Mannite-nitrate agar, 3 weeks.

Fig. 82.—Sporulation replaced by gonidangia formation. *A. chroococcum* (No. 20). Potato, 1 week.

Fig. 83.—Beginning endosporulation of large rods. *A. agile* (No. 16c). Beef agar, 3 days.

Fig. 84.—Regeneration of large globular cells from symplasm. *A. chroococcum* (No. 25). Potato, 1 week.

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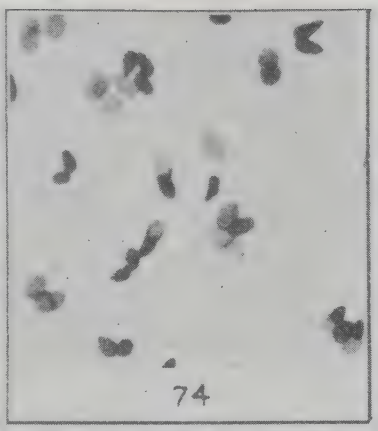
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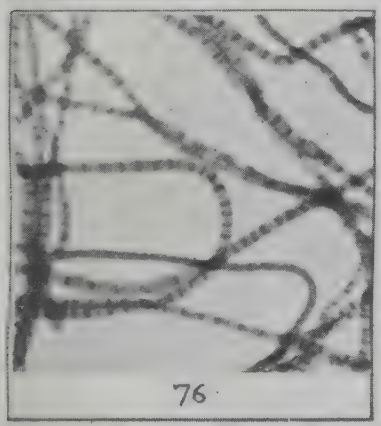
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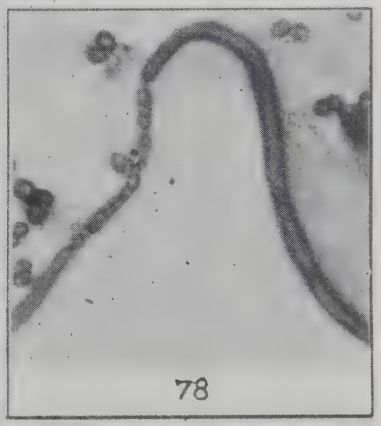
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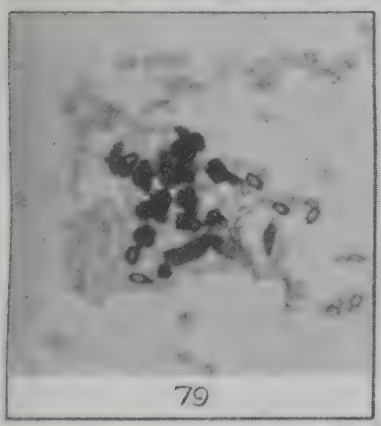
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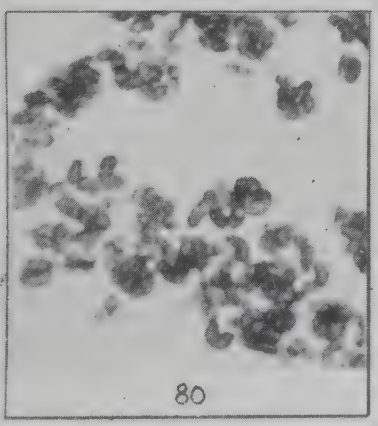
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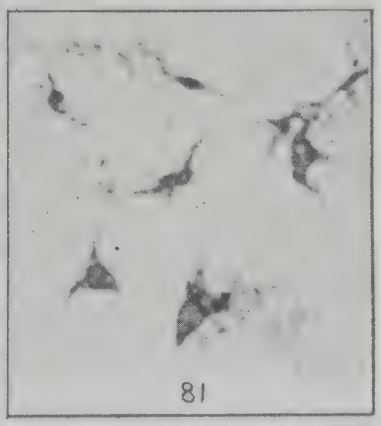
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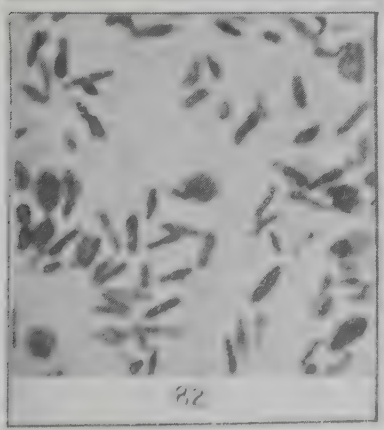
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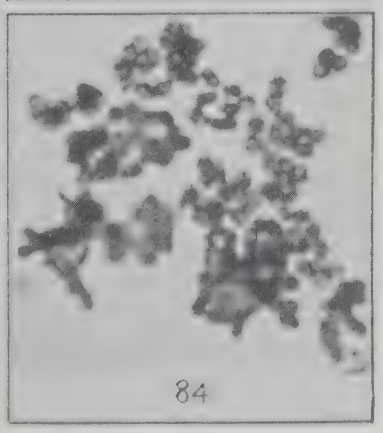
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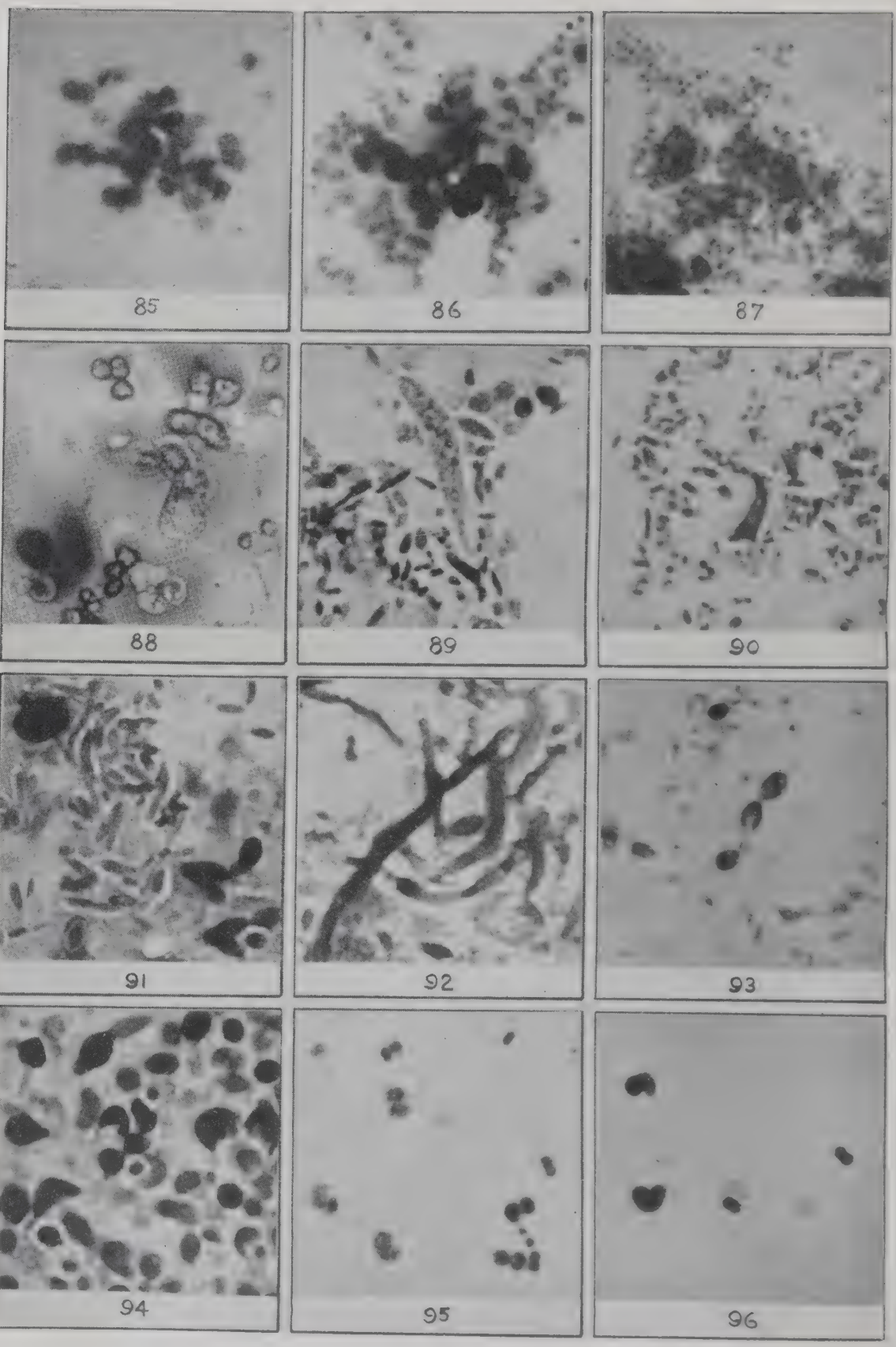
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PLATE 8

Gonidangia; conjunction.

Fig. 85.—Typical globular gonidangia. *Azotobacter chroococcum* (No. 22). Beef agar, 3 weeks.

Fig. 86.—Dissolution of gonidangia. *A. chroococcum* (No. 22). Beef agar, 1 week.

Fig. 87.—Dissolution of gonidangia. *A. Beijerinckii* (No. 4). Mannite-nitrate agar, 9 days.

Fig. 88.—Dissolution of gonidangia. *A. chroococcum* (No. 12). One percent salt-mannite-nitrate agar, 4 days.

Fig. 89.—Globular and threadlike gonidangia; also conjunction. *A. chroococcum* (No. 10). Mannite-nitrate agar, 4 days.

Fig. 90.—Regeneration of cells in gonidangium. *A. chroococcum* (No. 25). Potato, 4 days.

Fig. 91.—Globular and threadlike gonidangia; also microcysts. *A. agile*(No. 16c). Beef agar, 2 weeks.

Fig. 92.—Threadlike gonidangia producing new cells. *A. agile* (No. 16). Potato agar, 1 week.

Fig. 93.—Microcysts germinating. *A. agile* (No. 16). Mannite-nitrate agar, 2 months.

Fig. 94.—Microcysts germinating, also liberating gonidia. *A. agile* (No. 7). Potato agar, 1 week.

Fig. 95.—Conjunction and fission. *A. vitreum* (No. 9). Potato, 4 days.

Fig. 96.—Same as figure 95.

PLATE 9

Symplasm.

Fig. 97.—Formation of symplasm by regenerative bodies. *A. chroococcum* (No. 23) Potato, 9 days.

Fig. 98.—Regenerative units starting to grow. *A. chroococcum* (No. 19). Beef gelatine, 4 weeks.

Fig. 99.—Regenerative bodies growing from symplasm. *A. chroococcum*(No. 25). Beef agar, 4 weeks.

Fig. 100.—Various cell forms growing from symplasm. *A. Beijerinckii* (No. 15). Mannite soil extract, 2 months.

Fig. 101.—Branching small cells growing from symplasm. *A. chroococcum* (No. 1). Mannite-nitrate solution, 5 days.

Fig. 102.—Sporulating small rods growing from symplasm. *A. Beijerinckii* (No. 15). Mannite-nitrate solution, 17 days.

Fig. 103.—Beginning formation of rods. *A. vitreum* (No. 9). Mannite-nitrate solution, 10 days.

Fig. 104.—Radial growth of rods from symplasm. *Bacillus fusiformis* Gottheil (No. 149). Beef agar, 2 weeks.

Fig. 105.—Tangential growth of rods from symplasm. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 106.—Formation of new cells by agglomeration of regenerative units. *A. chroococcum* (No. 22). Mannite soil extract, 4 days.

Fig. 107.—Formation of filidia by agglomeration. *A. Beijerinckii* (No. 15). Mannite soil extract, 8 months.

Fig. 108.—Formation of sclerotia. *A chroococcum* (No. 19). Potato, 4 weeks.

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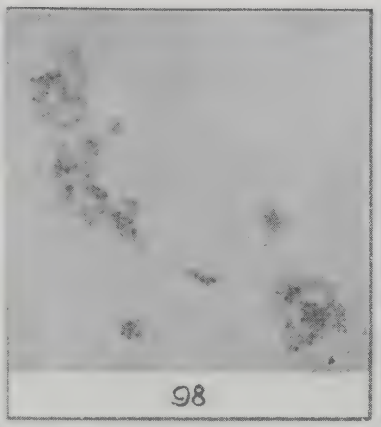

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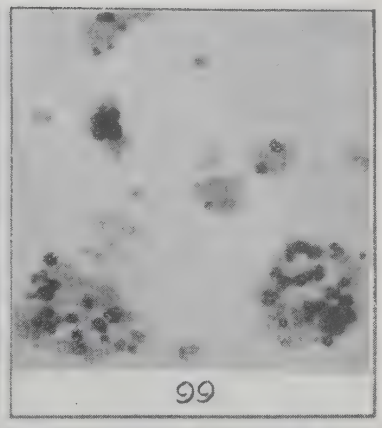

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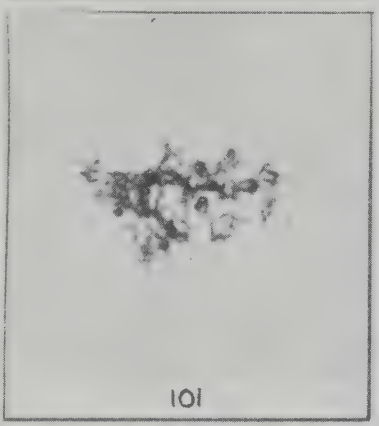

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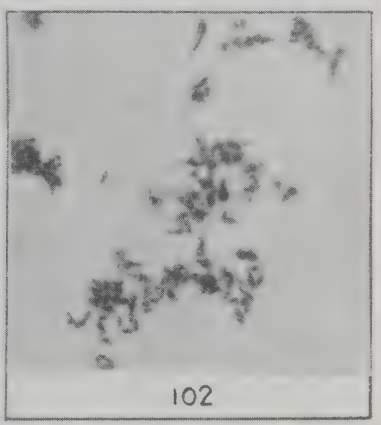

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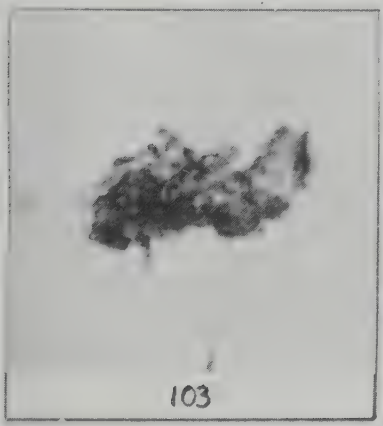

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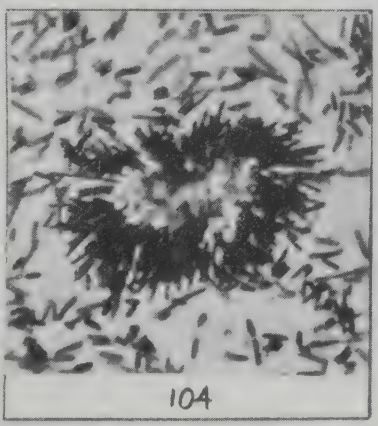

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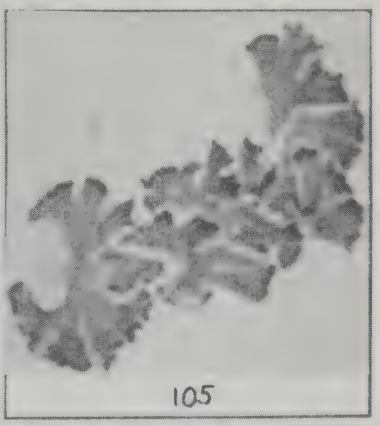

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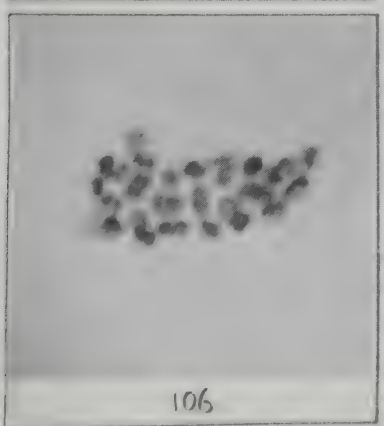

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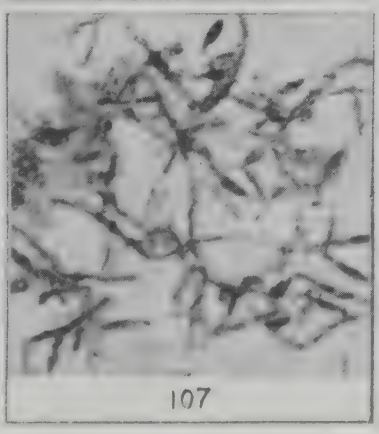

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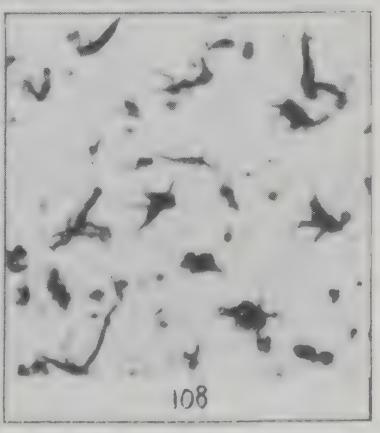

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INFLUENCE OF FERTILIZERS CONTAINING BORAX ON THE GROWTH AND FRUITING OF COTTON[1]

By J. J. SKINNER, *Biochemist, Soil-Fertility Investigations, Bureau of Plant Industry,* and F. E. ALLISON, *Soil Biochemist, Fixed Nitrogen Research Laboratory, United States Department of Agriculture*

The injury to cotton and other crops during the season of 1919 by fertilizers containing borax led to considerable experimentation, and a number of reports have been made recording the results of these studies on the effect of borax and fertilizers containing borax on crops, especially on corn, cotton, and potatoes. The extent of the injury to cotton and potatoes in 1919 is described by Schreiner, Brown, Skinner, and Shapovalov (*8*)[2] in a report published in 1920. Other reports on the injury to cotton have been made by Blackwell and Collings (*1*) and Plummer and Wolf (*7*). Conner (*4, 5*) first investigated the effect of borax on plant growth; his work was with corn, although later he also studied its effects on several other plants. Morse has reported upon the effects of borax in fertilizer in Maine (*6*).

In order to study the matter fully, a series of field experiments planned by the Office of Soil-Fertility Investigations of the Bureau of Plant Industry, United States Department of Agriculture, was conducted in 1920 on several types of soil and on several crops. Arrangements were made to conduct these experiments at Presque Isle, Me., cooperatively with the Maine Agricultural Experiment Station; at New Brunswick, N. J., cooperatively with the New Jersey Agricultural Experiment Station; at Muscle Shoals, Ala., cooperatively with the Fixed Nitrogen Research Laboratory, then of the War Department; and at the Arlington Experimental Farm, Va.

Potatoes were grown in Maine, potatoes and corn in New Jersey, cotton and corn in Alabama, and potatoes, corn, Lima beans, snap beans, and cotton at the Arlington Experimental Farm. The results obtained with potatoes and corn in New Jersey by Blair and Brown (*2*) have been given in a previous publication and a report of the work conducted by Brown (*3*) with potatoes in Maine has recently appeared.

In the present article the results obtained with cotton at Arlington Experimental Farm, Va., and at Muscle Shoals, Ala., are presented. The plan of the experiment involved the use of a fertilizer analyzing 4 per cent NH_3, 8 per cent P_2O_5, and 4 per cent K_2O, which was applied to cotton at the rate of 1,000 pounds per acre. This fertilizer was made from acid phosphate, muriate of potash, sodium nitrate, ammonium sulphate, and cottonseed meal. The fertilizer free from borax served as a control. Borax was mixed with this fertilizer in such proportions as to make the applications of anhydrous borax 5, 10, and 20

[1] Accepted for publication Aug. 24, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 443.

pounds per acre. In order to determine the effect of the rainfall and weather conditions on the action of the borax, the experiment was repeated at both places several times. The first application and planting were made early in June, and again at intervals of about a week. Six of these tests were made at Arlington and six at Muscle Shoals. It was realized that cotton planted at as late a date as this would not fully mature in either locality, but it was considered desirable to have some information concerning the action of borax on this crop, although the season was already advanced. The injury by borax has been shown to be principally to the young plant and in the early period of its growth. The experiments were therefore undertaken, and the effects on germination, on growth, and on boll formation were noted and recorded.

RESULTS OBTAINED AT ARLINGTON, VA.

The experiments conducted on the Arlington Experimental Farm were on a rich, silty loam soil, admirably suited for truck and general farm crops. The land is level and uniform and is tile drained.

The plan of the experiments involved the application of the fertilizers in the seed drill and by broadcasting; also the planting of the seed immediately after applying the fertilizers as well as after an interval of a week. A single row 132 feet long was used for each treatment. To one-third of the row, 44 feet, the fertilizer was applied in the drill and the seed was planted one week later. This is shown in section 1 of Table I. In section 2, which is the second 44-foot length, the fertilizers were applied in the drill as in section 1, and the seed was planted immediately. On the third section of the row the fertilizers were sown broadcast and the seed was planted immediately. Where the fertilizer was put in the drill it was mixed with the soil by raking with a hoe and was covered with an inch or two of dirt before the seeds were planted. In the broadcasted section the area was raked to mix the fertilizers. Each treatment, or plot, covered an area of 1/270 acre. The cotton was thinned to 45 hills per plot and two plants in each hill. Fertilizer analyzing 4 per cent NH_3, 8 per cent P_2O_5, and 4 per cent K_2O, was used and applied at the rate of 1,000 pounds per acre. In one row the fertilizer without borax was used as a control; in the second row the fertilizer containing borax in sufficient quantity to apply 5 pounds of anhydrous borax per acre was used; and in the third row the fertilizer contained sufficient borax to add 10 pounds per acre. In some of the tests 20 pounds of borax per acre were used. In order to determine the influence of weather conditions on the effect of borax on cotton, the experiments were repeated six times; the first series (A) was begun on June 2, the second (B) on June 9, the third (C) on June 18, the fourth (D) on July 7, the fifth (E) on July 15, and the sixth (F) on August 3. Notes were taken during the summer, and the plants in each plot were measured for height when several weeks old. This was done by measuring a large number of plants and taking the average. A record of the number of bolls and squares that formed was made. The plants were cut on October 1 and the green weights were taken and recorded. The complete data are given in Table I.

Effect of borax on the growth and fruiting of cotton grown in a productive silty loam soil at Arlington farm, Va., in 1920 a

Experiment series, date of fertilizer application, and quantity of borax applied per acre.	Fertilizers applied.														
	Sec. 1.—In drill and seed planted one week later.					Sec. 2.—In drill and seed planted immediately.					Sec. 3.—Broadcast and seed planted immediately.				
	Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.	Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.	Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.
Series A, June 2:	*Inches.*	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*	*Inches.*	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*	*Inches.*	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*
No borax	16.9	61	.....	1,680	.....	12.5	60	.....	1,777	.....	14.8	73	.....	1,435	.....
5 pounds	12.4	56	− 8.2	1,295	−22.9	11.6	59	− 1.7	1,000	−43.7	14.1	80	+ 9.6	1,252	−12.7
10 pounds	11.2	58	− 5	1,315	−21.7	10.1	61	+ 1.7	967	−45.6	11.0	55	−24.7	901	−37.2
Series B, June 9:															
No borax	8.8	67	.....	1,478	.....	11.5	72	.....	1,441	.....	13.0	72	.....	1,525	.....
5 pounds	8.1	69	+ 3	1,400	− 5.3	7.3	62	−14	1,305	− 9.4	10.0	79	+ 9.7	1,503	− 1.4
10 pounds	6.9	67	0	1,302	−11.9	6.5	58	−19.5	1,361	− 5.6	8.8	68	− 5.6	1,600	+ 5
Series C, June 18:															
No borax	9.6	62	.....	1,500	.....	10.2	69	.....	1,697	.....	10.7	68	.....	1,692	.....
5 pounds	7.0	62	0	1,590	+ 6	9.6	70	+ 1.4	1,750	+ 3.1	9.1	71	+ 4.4	1,800	+ 6.4
10 pounds	6.7	57	− 8	1,490	− .7	8.7	72	+ 4.4	1,505	−11.3	7.8	64	− 5.9	1,613	− 4.7
20 pounds	6.5	56	− 9.7	1,380	− 8	6.8	49	−29	1,480	−12.8	7.2	53	−22	1,520	−10.2
Series D, July 7:															
No borax	.....	40	.....	1,100	.....	.....	45	.....	1,539	.....	.....	54	.....	1,610	.....
5 pounds	.....	37	− 7.5	1,000	− 9.1	.....	49	+ 8.9	1,392	− 9.5	.....	51	− 5.6	1,630	+ 1.2
10 pounds	.....	29	−27.7	760	−30.9	.....	33	−26.7	1,050	−31.7	.....	41	−24.2	1,210	−24.8
20 pounds	.....	21	−47.5	597	−45.7	.....	29	−35.5	930	−39.5	.....	35	−35.2	1,030	−36
Series E, July 15:															
No borax	.....	26	.....	622	.....	.....	28	.....	823	.....	.....	35	.....	1,146	.....
5 pounds	.....	26	0	680	+ 9	.....	24	−14.3	822	− .1	.....	36	− 2.9	1,137	− 0.8
10 pounds	.....	21	−19.2	585	− 6	.....	23	−18	718	−12.7	.....	33	− 5.8	1,005	−12.3
20 pounds	.....	21	−19.2	560	−10	.....	18	−36	434	−47.2	.....	30	−14.3	1,020	−11
Series F, August 3:															
No borax	.....	10	.....	0	.....	.....	13	.....	0	.....	.....	17	.....	0	.....
5 pounds	.....	10	0	0	.....	.....	11	−15.4	0	.....	.....	14	−17.6	0	.....
10 pounds	.....	8	−20	0	.....	.....	12	− 7.7	0	.....	.....	14	−17.6	0	.....
20 pounds	.....	9	−10	0	.....	.....	8	−38.4	0	.....	.....	13	−23.5	0	.....

[1] The measurements were made in experiments A and B, sections 2 and 3, on July 27; in section 1, August 3; in experiment C, sections 2 and 3, on August 3; and in section 1, on August 10.

An examination of the data given in Table I shows generally that the growth of the cotton was checked and the fruiting decreased by the borax. The degree of injury, however, varies with the different plantings and with the different methods of applying the fertilizers. The germination was rather irregular where 20 pounds of borax were applied, and when the cotton was young some died in spots. Ten pounds had a decided effect on the color of the foliage in each experiment. Where borax was used the foliage was much lighter green.

EFFECT UNDER DIFFERENT METHODS OF APPLYING FERTILIZERS

In section 3, where the fertilizers were sown broadcast and 5 pounds of borax applied per acre, no injury was observed in series A, B, and C, and only a slight reduction in growth in series D, E, and F. There was only a slight reduction in the fruiting of the plants. With 10 pounds of borax there was a decided decrease in growth and in the number of bolls formed. The degree of harmfulness produced by borax in the different plantings is noticeable. It is probably due to weather conditions to be considered later. Where 20 pounds of borax were used there was a decided harmful effect. In series C, D, E, and F there was a reduction in growth of 22, 35.2, 14.3, and 23.5 per cent, respectively, and a reduction in boll formation of 10.2, 36, and 11 per cent in series C, D, and E, respectively. In section 2, where the fertilizers were applied in the drill and the seed was planted immediately afterwards, the harmfulness of borax when 10 or 20 pounds per acre were used was marked. The growth was checked more in section 2 than where the fertilizers were sown broadcast. Five pounds per acre reduced growth considerably in series E and F. In section 1, where the planting was not made until one week after the fertilizers were applied and where in each case a rain intervened, the harmfulness of the borax is considerably less than in sections 2 and 3, except in series D and E. To judge from the data as a whole, it can hardly be concluded that 10 pounds were harmful when applied as in this section of the experiments.

A record of the height of the plants made when the crop was young shows that the growth was checked in the very beginning by the borax. In plate 1, A, is shown the effect of borax on the young plants in series A. In the foreground the seed was planted in the drill immediately after applying the fertilizer, and in the back half of the row the fertilizers were sown broadcast. The broken stand is readily discernible in the row receiving 10 pounds of borax. The cotton had not been thinned to a stand when the photograph was made. The young plants died in spots soon after emerging to the surface. In plate 1, B, the surviving plants are shown after having made considerable growth. It is seen here that the 10-pound borax row is much smaller than the control and the 5-pound borox row. In plate 2, A, is shown the cotton in series D. Here the broken stand in the last row, which has 20 pounds of borax, is shown.

INFLUENCE OF RAINFALL

In order to study the influence of rainfall on the effect of borax on the crop, the weekly record of the rainfall together with the maximum and minimum temperature at the Arlington Experimental Farm during the period of the experiments is given in Table II.

TABLE II.—*Temperature and rainfall at Arlington Farm, Va., June to September, 1920*

Week of—	Rainfall.	Temperature.		Week of—	Rainfall.	Temperature.	
		Maximum.	Minimum.			Maximum	Minimum.
	Inches.	*° F.*	*° F.*		*Inches.*	*° F.*	*° F.*
May 30 to June 5	1.19	90	47	Aug. 1 to 7	0.71	87	53
June 6 to 12	1.08	95	51	Aug. 8 to 14	1.13	92	66
June 13 to 19	.36	93	57	Aug. 15 to 21	2.57	91	66
June 20 to 26	1.68	84	56	Aug. 22 to 28	.29	85	62
June 27 to July 3	1.96	94	57	Aug. 29 to Sept. 4	.11	90	48
July 4 to 10	.62	89	55	Sept. 5 to 11	2.01	84	52
July 11 to 17	.41	91	63	Sept. 12 to 18	.01	87	45
July 18 to 24	1.19	95	59	Sept. 19 to 25	.03	90	44
July 25 to 31	.80	92	53	Sept. 26 to Oct. 2	1.43	88	55

Comparing the rainfall record at or about the time the fertilizers were applied in the various experiments, it is seen that the moisture conditions were about optimum when and after the plantings were made in series A, B, and C. The rainfall, however, was very light during the weeks of July 4 and July 11 and was again light the weeks of July 25 and August 1. The effect of the borax in experiments D and E, which were planted in the period of dry weather, is more severe than in the experiments which were planted when the moisture was normal. For example, in section 1, 20 pounds of borax per acre reduced the growth 9.7 per cent in series C, 47.5 per cent in series D, and 19.2 per cent in series E. In section 2, the growth was reduced in series C 29 per cent; in D 35.5 per cent, and in E 36 per cent. In section 3, the growth was reduced 22 per cent in series C, 35.2 per cent in D, and 14.3 per cent in E. The formation of bolls was also reduced more in series D and E than in C. A few days after the plantings were made in the latter experiments a light rain fell, which was followed by a dry period. While the plants were young in the earlier experiments there were occasional heavy rains, and at no time did the soil become very dry. It is not probable that a rainfall of 1.08 to 1.68 inches in one week distributed over a period of several days would wash very much borax out of the reach of the roots of the cotton. However, it would result in the diffusion of the borax through the soil, and this diffusion could undoubtedly account for the lesser degree of injury in series A, B, and C. Under the rainfall conditions of series D and E the borax was concentrated in locations surrounding the roots of the young plants and would naturally cause a more severe injury and a greater retardation of growth.

The data as a whole show that the effect of borax on cotton under the weather conditions prevailing at the time of this test is decidedly harmful when 20 pounds per acre are applied in the drill or sown broadcast. This quantity showed harmful effects whether the seed was planted immediately after the fertilizers were applied or after the intervening of a light rain. When applied in the drill, 10 pounds per acre checked growth decidedly, but were only slightly harmful when sown broadcast.

RESULTS OBTAINED AT MUSCLE SHOALS, ALA.

PLANTINGS ON CLARKVILLE LOAM

The experiments at Muscle Shoals were made on soil of Clarkville loam, located on a gentle slope, and well drained. The soil is fairly retentive of moisture and does not become compact. The plan of the experiments differed somewhat from that at the Arlington Experimental Farm in that the fertilizer was applied only in the drill and the seed was planted immediately as in section 2 of the Arlington tests. The 4–8–4 fertilizer was used at the same rate of application (1,000 pounds), and borax was used so as to apply 5, 10, and 20 pounds of anhydrous borax per acre. Two controls in which no fertilizer was used were added. One row 100 feet long was used for each treatment, which makes each plot approximately 1/110 acre. The test was repeated six times. The first was started June 12 and the others followed at intervals of about one week. The separate plantings are designated as series A, B, C, D, E, and F. Cleveland Big Boll cotton was used.

In Table III are given the data for this set of plots, including the height of the cotton plants at intervals during growth, the number of bolls which formed, and the green and dry weights of the plants, including the roots.

TABLE III.—*Effect of borax in fertilizers on the growth of cotton, on Clarkville loam at Muscle Shoals, Ala., in 1920*

Experiment series, date of starting, and quantity of borax applied per acre.	Average height of plants.			Bolls on Oct. 26.	Green weight of plants.	Increase or decrease.	Dry weight of plants.
	July 28.	Aug. 20.	Oct. 18.				
Series A, June 12:	*Inches.*	*Inches.*	*Inches.*	*Number.*	*Pounds.*	*Per cent.*	*Pounds.*
No fertilizer	.....	12.3	28.8	237	31	.....	10
No borax	12.9	25.3	45.5	667	93	.....	36
5 pounds	9.6	21.8	44.4	583	86	− 7.6	33½
10 pounds	7.1	17.3	42.1	456	85	− 9.4	32
20 pounds	4.9	13.2	32.6	242	44	−52.7	15
Series B, June 19:							
No borax	12.0	28.6	50.0	716	104	.....	40
5 pounds	10.5	25.7	43.3	563	84	−19.2	34½
10 pounds	8.0	24.1	43.0	576	78	−25.0	31½
20 pounds	6.9	15.6	39.1	383	63	−39.4	25
Series C, June 26:							
No borax	9.4	18.1	45.2	337	86	.....	34
5 pounds	6.3	15.1	40.5	220	78	− 9.3	28½
10 pounds	6.9	12.9	39.9	224	75	−12.8	26
20 pounds	5.0	9.6	31.5	97	43	−50.0	15
Series D, July 3:							
No borax	6.2	16.1	42.2	112	63	.....	25½
5 pounds	5.2	15.1	41.6	94	62	− 1.6	24
10 pounds	4.1	12.0	32.9	92	59	− 6.4	23
20 pounds	3.2	7.2	29.3	53	39	−38.1	13
Series E, July 11:							
No borax	.....	12.3	36.8	45	55	.....	17½
5 pounds	.....	11.5	33.7	48	56½	+ 2.7	20
10 pounds	.....	9.9	31.3	46	50½	− 8.2	20
20 pounds	.....	8.4	26.1	14	38	−31.0	11
Series F, July 20:							
No fertilizer	.....	5.1	9.5	0	5½	.....	1
No borax	.....	10.0	28.2	5	46½	.....	15
5 pounds	.....	8.9	29.1	7	47	+ 1.0	15
10 pounds	.....	8.7	25.7	4	41	−11.9	11
20 pounds	.....	7.6	22.4	1	35	−24.8	8

Table IV records the weekly rainfall and temperatures at Florence, Ala., 2 miles from United States Nitrate Plant No. 2, so that the relation of rainfall to the degree of harmfulness of borax in the different series of the Muscle Shoals experiments may be correlated.

TABLE IV.—*Temperature and rainfall at Florence, Ala., June to September, 1920*

Week of—	Rainfall.	Temperature.		Week of—	Rainfall.	Temperature.	
		Maximum.	Minimum.			Maximum.	Minimum.
	Inches.	*° F.*	*° F.*		*Inches.*	*° F.*	*° F.*
June 6 to 12	0	97	53	August 1 to 7	0	94	60
June 13 to 19	.52	98	63	August 8 to 14	5.10	90	66
June 20 to 26	.58	92	56	August 15 to 21	4.46	91	64
June 27 to July 3	.08	93	61	August 22 to 28	.59	91	56
July 4 to 10	.76	94	62	August 29 to September 4	.42	96	61
July 11 to 17	.98	93	61	September 5 to 11	2.17	93	55
July 18 to 24	1.50	98	63	September 12 to 18	.32	93	55
July 25 to 31	.06	95	57	September 19 to 25	.54	92	55
				September 26 to October 2	0	92	37

From the results of series A it will be observed that 5 pounds of borax per acre produced some injury to cotton when applied in the row. Larger quantities showed even greater toxic effects. From the figures showing the average height of plants it will be seen that the injury was the greatest when the plants were small. A marked recovery followed, but the initial retarding was not entirely overcome. The green weight of plants was decreased 7.6 per cent by 5 pounds of borax per acre and 52.7 per cent by 20 pounds. The boll formation was decreased in about the same proportion or to an even greater extent, and in addition maturity was delayed. The cotton in series A was planted when the soil was too dry to germinate the seed. On June 18 a light shower fell, approximately 0.34 of an inch, which was sufficient to cause germination, and for three weeks following but little rain fell. As seen in Table IV, 0.52 of an inch of rain fell the week of June 13, 0.58 of an inch the week of June 20, and 0.08 of an inch the week of June 27. The season for the three weeks following the planting of the cotton in series A was rather dry.

The cotton in series B was planted just subsequent to a light shower and germinated in minimum time. The rainfall during the early period of growth was rather light. In the first week after planting 0.58 of an inch of rain fell, the second week 0.08 of an inch, and the third week 0.76 inch. The growth was about the same as in series A, except that in series B, 5 pounds of borax caused a relatively greater injury and 20 pounds less injury than in series A. The same general tendency prevailed, however, and the same visible effects on the young plants were in evidence. The retarding effects of the borax continued throughout the growing season.

At the time of planting series C the soil was very moist, but for more than a week after planting no rain fell and the soil became very dry. In

the second week after planting 0.76 of an inch of rain fell, which thoroughly moistened the ground. The amount of injury produced by the different quantities of borax corresponded very closely to the results in series B.

The planting of series D was made when the soil was very dry, and the seed did not germinate immediately. A good rain occurred about one week later, and about 0.76 of an inch fell; the following week there was 0.98 of an inch precipitation, and the third week 1.5 inches. After the germination of the seed there was more rainfall, and the soil was consequently more moist than when the cotton was young in the plots planted earlier. The injuries attributable to the borax were scarcely as extensive in this series as on the plots planted earlier, but they were in the same order. In series D 5 pounds of borax reduced the green weight 1.6 per cent, 10 pounds 6.4 per cent, and 20 pounds 38.1 per cent. In series C the reduction was, respectively, 9.3, 12,8, and 50 per cent; in series B, 19.2, 25, and 39.4 per cent; and in A, 7.6, 9.4, and 52.7 per cent.

In series E the cotton was planted when the soil was moist and in good tilth. Several days after planting 0.98 of an inch of rain fell, which was followed by a period of one week without rain. This period was followed by several days of rain, and, as seen in Table IV, during the week beginning July 18 1.5 inches of rain fell. The toxic effects were not as marked as on the plots planted earlier. There was a slight stimulation where 5 pounds of borax per acre were used, 10 pounds reduced the growth 2.7 per cent, and 20 pounds 31 per cent.

Series F was planted very late, and growth continued only about two months. The data are given in the table, however, and it will be seen that the effects of the borax were of about the same magnitude as in series E.

In Plate 3 the effect of borax is shown on the cotton in series A, planted June 12. The views shown at A, B, and C were photographed on July 29, August 8, and September 18, respectively, the last date being shortly before the plants were cut and weighed. Plate 3, A, was photographed before the cotton was thinned; the unbroken stand and uneven appearance of the row receiving 20 pounds of borax per acre is shown.

In Plate 4, A and B, are shown the effects of borax in series D. View A of Plate 4 was photographed on August 23 and B on September 18. In Plate 4C, the cotton in series F is shown.

PLANTINGS ON COLBERT SILT LOAM

Other experiments with cotton were begun at Muscle Shoals, Ala., similar to those at Arlington Farm, Va., and to those in New Jersey with potatoes and corn. These included immediate planting of seed after applying fertilizer, delayed planting, and broadcasting. The quantities of borax used varied from 1 to 400 pounds per acre. Two rows 70 feet long were used for each treatment on the Colbert silt loam, which is a heavier soil than the Clarkville loam. The fertilizers were applied on May 10. The rainfall for the month prior to starting the experiments, and for a like period afterwards, was exceedingly heavy. The soil became very compact from the excessive rains, resulting in a very poor stand over the entire area. The experiments were continued, however, in order to observe the effects of the borax; but a harvest was not made, as the broken stand appeared to make it useless.

Borax caused the greatest injury to cotton in the early stages, either preventing germination or in lesser amounts merely retarding growth and preventing chlorophyll formation. A record of observations made three weeks after planting is given in Table V.

TABLE V.—*Effect of varying quantities of borax on the growth of cotton on Colbert silt loam at Muscle Shoals, Ala., in 1920*

[Observations were made three weeks after planting.]

Quantity of borax applied per acre.	Fertilizers applied on May 10.		
	Sec. 1.—In the row and seed planted 10 days later.	Sec. 2.—In the row and seed planted immediately.	Sec. 3.—Broadcast and seed planted immediately.
No borax.			
1 pound	Normal	Normal	Normal.
2 pounds	do	do	Do.
3 pounds	do	do	Do.
No borax.			
4 pounds	Normal	Normal	Do.
5 pounds	do	do	Do.
10 pounds	do	do	Do.
No borax.			
20 pounds	Slight retarding	Slight injury	Slight retarding.
30 pounds	Somewhat stunted	Plants small; many dying.	Do.
50 pounds	Germination low; plants show yellowing.	Germination low; plants dying.	Somewhat stunted.
No borax.			
100 pounds	Germination about 50 per cent; plants dying.	Only an occasional seed germinated; plants, dying.	Germination decreased and plants dying.
200 pounds	Only an occasional seed germinated; plants about dead.	Seven seeds germinated; plants about dead.	Germination decreased about 70 per cent; most plants dead.
400 pounds	No germination	No germination	Twelve seeds germinated and plants died.
No borax.			

The quantity of borax required to produce a noticeable injury to cotton receiving fertilizer in the row was 20 pounds. To lower germination appreciably and cause the death of any very large percentage of the plants 50 pounds were necessary. Where the fertilizer was used in the row and planting was delayed for 10 days the injuries seemed to be decreased about 50 to 75 per cent. Distributing the fertilizer broadcast, as in section 3, decreased the effects as much or possibly slightly more than delaying planting. It is shown that any method of use which decreased the concentration of the borax around the plant roots markedly decreases the injuries.

During the 10 days preceding the planting, May 1 to 10, 2.06 inches rain fell, and for the 10-day period following the planting 3.34 inches rain fell. On the second day after planting 1.6 inches precipitation occurred, which was followed by light showers for several days. On the seventh day after planting there was a rainfall of 1.56 inches. The total rainfall for the month was 5.70 inches.

Even with this heavy rainfall there was unquestionable injury from the borax with 30 pounds per acre. With 50 pounds per acre germination was low when the fertilizer was put in the drill and seed planted immediately, and many of the plants died after germinating. When the fertilizer was sown broadcast the plants were stunted. With 100 pounds and over per acre there was practically no germination. The section of the field where 100, 200, and 400 pounds of borax per acre were used is shown in Plate 2, B.

SUMMARY

Experiments were made to test the effect of borax in fertilizers on cotton at Arlington Farm, Va., and at Muscle Shoals, Ala. The soil at Arlington Farm is a productive silt loam suitable for truck and general farm crops. The soils on which the experiments were located at Muscle Shoals were the Clarkville loam and the Colbert silt loam. Borax was mixed with fertilizers and applied so as to add 5, 10, and 20 pounds of anhydrous borax per acre in two of the experiments. In a third test the quantities varied from 1 to 400 pounds per acre.

At Arlington Farm and Muscle Shoals borax in small quantities was injurious to the young plants.

On the silt loam at Arlington Farm, when the fertilizer was applied broadcast, 5 pounds of borax per acre were not always injurious, 10 pounds were slightly injurious, and 20 pounds were distinctly so. Application of 20 pounds reduced the weight of the green plants in the various experiments from 15 to 35 per cent. When the fertilizer was applied in the drill and seed planted immediately, 5 pounds was slightly harmful, 10 pounds was distinctly injurious, and 20 pounds caused severe injury, especially on germination and early growth. When the fertilizer was applied and allowed to stand until after a rain before the seed were planted, the effect was less severe.

On the Clarksville loam at Muscle Shoals a somewhat similar result was obtained. In one of the experiments, 5 pounds of borax applied in the drill with the fertilizer decreased the green weight of the plants 5.7 per cent, 10 pounds decreased the weight 12 per cent, and 20 pounds decreased it 39 per cent. The injuries to early growth were much more marked than these figures would indicate.

The influence of rainfall was noted. While the season was a normal one from June, the rainfall materially influenced the effect of the borax. Wherever a light rainfall occurred soon after planting and was followed by a dry period the effect was severest. If heavy showers followed periodically after planting the effect was less severe.

In an experiment started in the early spring on the Colbert loam at Muscle Shoals where the rainfall was very heavy for 10 days after planting, germination was materially affected where the fertilizers were applied broadcast by 100 pounds of borax per acre, and no germination took place with 200 pounds per acre. Where the fertilizers were put in the row, germination was materially affected by 50 pounds per acre and was prevented entirely by 100 pounds.

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PLATE 1

Effect of borax on cotton in experiment A at the Arlington Experimental Farm.

A.—0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre. Photographed July 10, 38 days after planting.

B.—Experiment A, five weeks later. Photographed August 17, 75 days after planting.

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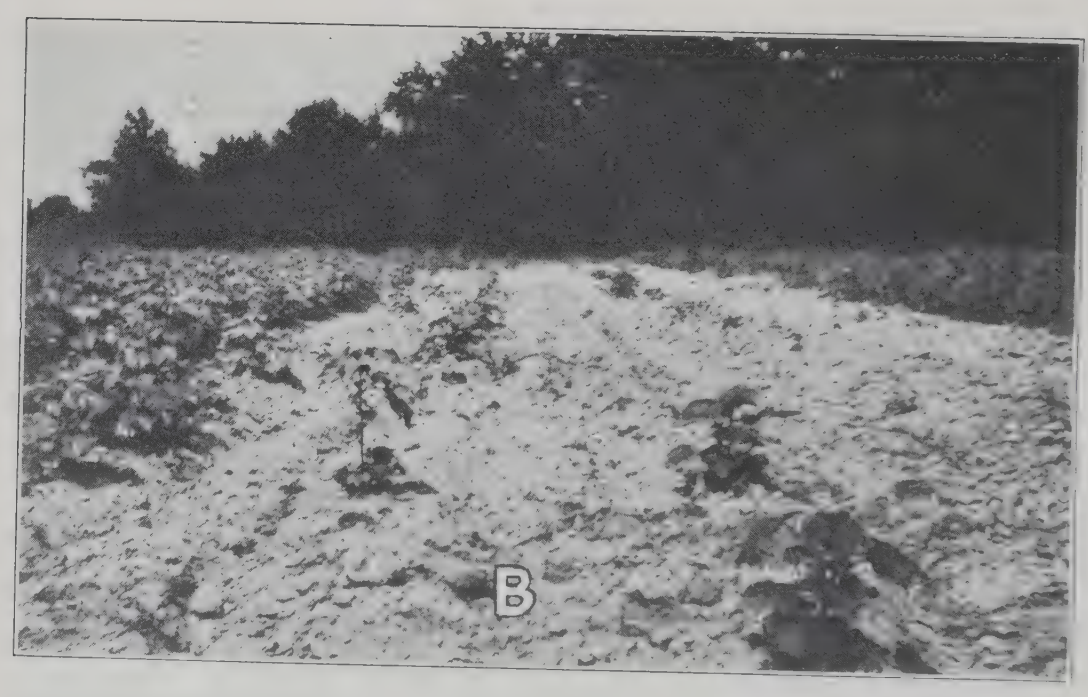

B

PLATE 2

Effect of borax on growth and germination of cotton.

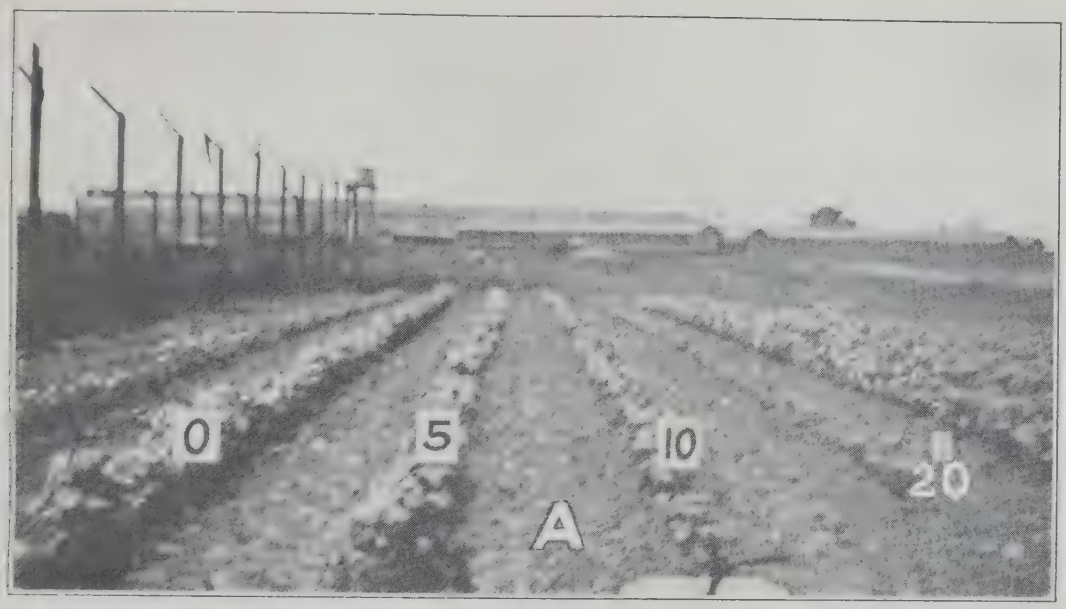
A.—Effect on cotton at the Arlington Experimental Farm in experiment C: 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre. Photographed 43 days after planting.

B.—Prevention of germination of cotton by 100, 200, and 400 pounds of borax per acre. The bare rows show where these large quantities of borax were used.

PLATE 3

Effect of borax on cotton in Section A in the Clarksville loam at Muscle Shoals, Ala. 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre.

A.—Photographed July 29, 47 days after planting.
B.—Photographed August 26, 74 days after planting.
C.—Photographed September 18, 96 days after planting.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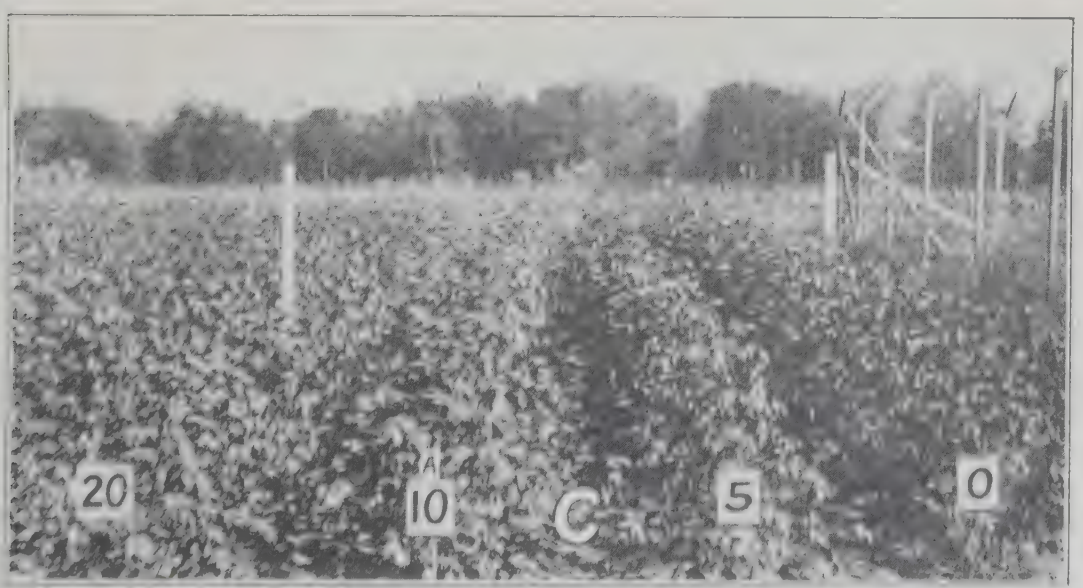
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PLATE 4

Effect of borax on cotton on Clarksville loam in Sections D and F. 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre.
A.—Cotton in Section D. Photographed August 23, 50 days after planting.
B.—Cotton in Section D. Photographed September 18, 75 days after planting.
C.—Cotton in Section F. Photographed September 18, 58 days after planting.

GENETICS OF BUNT RESISTANCE IN WHEAT [1]

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INTRODUCTION

The losses due to bunt, *Tilletia tritici* (Bjerk.) Wint., in the Pacific Northwest have been steadily increasing for the past 25 years, notwithstanding the most earnest efforts on the part of scientists and farmers to reduce them. The seed has practically always been treated with blue vitriol or formaldehyde, but in spite of every precaution the winter wheat often contains from 10 to 50 per cent of bunted heads at harvest time, apparently due to soil infection from wind-borne spores. In the State of Washington alone the most conservative estimates place the losses at more than 1,000,000 bushels of wheat annually. The bunt problem has caused, and is causing, more interest and anxiety than any other of like nature through the winter wheat districts. Spring-sown wheat seldom produces a bunted crop if the seed has been carefully disinfected, the spores in the soil having perished during the winter.

The work has been closely linked with the other cereal and pathological investigations at the Washington Agricultural Experiment Station, and many friends and coworkers have contributed to the material in hand. Acknowledgment is here made to the various members of the staffs of the Washington Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for their ever-ready help and enthusiasm; to E. M. East for helpful suggestions and criticism of the manuscript, and also to H. B. Humphrey, H. M. Woolman, E. G. Schafer, and F. D. Heald for suggestions and corrections in the presentation of the subject matter.

When the writer became assistant cerealist of the Washington Agricultural Station in 1911, many inquiries were coming in from farmers concerning control measures for bunt in winter wheat. The limitations of seed treatment were well known, and soil sterilization seemed impracticable under the extensive system of wheat raising on the great farms of the winter-wheat belt. The possibility of obtaining or developing a strain that would resist the attacks of the fungus and at the same time fulfill the requirements of winter hardiness, prolificacy, milling quality, etc., seemed the most promising solution of the problem. Accordingly hundreds of varieties from the principal wheat districts of the world have been introduced and many hybrids have been made in an attempt to find or develop such a wheat. Preliminary reports (*13, 14*)[2] of this work have already been published.

The following paper deals with that part of the investigation concerning the comparative resistance of different wheats and the inheritance of the factors that cause resistance, as expressed in the hybrid segregates of succeeding generations.

[1] Accepted for publication August 31, 1921. Submitted to the faculty of the Bussey Institution of Harvard University in partial fulfillment of the requirements for the degree of doctor of science, April 29, 1921.

[2] Reference is made by number (italic) to "Literature cited," p. 476–479.

RESISTANCE PHENOMENA IN GENERAL

RELATIONSHIPS AND RESPONSES OF HOST AND PARASITE

It has long been known that plants and animals vary greatly in their susceptibility to disease, and that the causal organisms are more or less restricted in their choice of host. This variation may be attributable to the inability of the causal organism to set up a pathogenic relationship with the host or to its inability to function after having established such relationship. Failure to infect may be due to an incompatible optimum of temperature, moisture, light, or nutrient materials. These factors may also provide the physiological basis for failure to function after infection has taken place. In addition, the outer coat may offer a mechanical obstruction. Thus, the bark of trees, the seed coats of grain, and the skins of animals offer a powerful protection against invading organisms which gain a ready access if injury occurs. However, a great majority of the pathogens that have been studied experimentally seem to have less difficulty in gaining entrance to closely related resistant forms than in continuing that existence after having gained entrance. Thus in Marryat's experiments (*32*) infection by yellow or stripe rust [*Puccinia glumarum* (Schm.) Erikss. and Henn.] occurred with equal readiness through the stomata of resistant and susceptible wheats. The susceptible Michigan Bronze wheat seemed to nurture the infection tubes and hyphae, the cells accepted the haustoria without shrinking, and normal spores were soon produced. Hyphae never seemed to flourish in the resistant Einkorn, but appeared stunted and watery and seldom produced haustoria. They soon died, but in dying they killed the host cells with which they came in contact—appearing to be mutually toxic. The American Club, intermediate in resistance, sometimes killed the fungus without reducing the vigor of the host cells, but in other places the hyphae were found flourishing while the host cells seemed to be dying. When spores did develop there was not enough force to break the epidermis. Apparently resistance in this case was produced by antitoxins furnished by the host and toxins furnished by the parasite. Ward (*60*), also working on the histology of resistance, showed that size of stomata, hairs, or comparative leaf surface had no influence whatever on the susceptibility of different bromes to brown rust, *Puccinia dispersa* Erikss. and Henn. After treating a mass of data statistically he drew the following conclusions:

> Resistance—is not to be referred to observable anatomical or structural peculiarities but to internal, i. e., intraprotoplasmic, properties beyond the reach of the microscope, and similar in their nature to those which bring about the essential differences between species and varieties themselves.

This was in 1902. Since then a great mass of experimental work in plant pathology, bacteriology, and medicine has added weight to his conclusions. For example, Stakman (*45*), who has done much work on the phenomena of resistance in cereals and grasses to the stemrust, *Puccinia graminis* Pers., concludes from his histological study of hyphal invasion (to quote his summary):

> 1. When plants practically immune to *Puccinia graminis* are inoculated, the fungus gains entrance in a perfectly normal manner.
> 2. After entrance the fungus rapidly kills a limited number of the plants cells.
> 3. The fungus, after having killed the host cells in its immediate vicinity, seems unable to develop further.
> 4. The relations between plant and parasite in partially resistant and almost wholly immune plants are different in degree only.

5. Hypersensitiveness of the host seems to be a common phenomenon not only among plants somewhat resistant to *P. graminis* but also among those almost totally immune to it.

These conclusions point to the probability that resistant and susceptible plants differ in the chemical composition of their cell walls or protoplasm or both. Valuable information might be gained along these lines by such investigations as the recent work of Rumbold (*43*) and Thiel and Weiss (*55*). Rumbold killed Chestnut trees by injecting an extract of the cankerous tissue caused by the chestnut-blight fungus [*Endothia parasitica* (Murr.) P. J. and H. W. Anderson] into the trunks of healthy trees. Extracts from healthy tissue produced no effect.

Thiel and Weiss were able to germinate teliospores of *Puccinia graminis tritici* Erikss. and Henn. in midwinter by soaking them 15 minutes in 1 per cent citric acid, although untreated spores would not germinate at all until spring. Other acids, lipoid solvents, and sodium hydroxid were ineffective. From this it would seem that the acid had acted as a specific activator through the cell wall on the protoplasm. Susceptible hosts may contain similar activators which stimulate the metabolism of the parasite.

The delicate chemotactic balance between host and parasite as an explanation of the differing degrees of resistance finds support in the investigations of Spinks (*44*) who found that nitrates, and especially lead and zinc nitrate, increased the susceptibility of wheat and barley to mildew and rust. Furthermore, potash and lithium salts decreased susceptibility; but resistant varieties maintained their relative resistance under either condition.

From this it is logical to assume that species or races which vary in their resistance to disease-causing organisms when growing under identical environmental conditions do so because of their physical and chemical individualism. If this be true, then the complex of causes responsible for resistance and immunity in plants is very similar to, if not identical with, those producing resistance and immunity in animals.

The classic researches the last 25 years in the antitoxin laboratories have shown that susceptibility to disease is caused by the physicochemical reactions of poisons, toxins, ptomains, etc., produced by the causal organism in the various tissues of the host. In acquired immunity the host cells have the power to develop counteracting chemicals which neutralize the harmful substances produced by the pathogene. In natural immunity either the counteracting chemicals are already present, the temperature, light, food, etc., are not optimum for the pathogene, or else the composition of the protoplasm of the host is such that no chemical reaction takes place. An illustration of the latter condition is described by Zinsser (*62*) regarding tetanus from the work of Blumenthal (*6, p. 185*). As is well known, the central nervous system is the special point of attack for tetanus poison, and Blumenthal found that tetanus toxin was neutralized by the brain tissue of susceptible animals, but the brain tissue of resistant animals like the chicken had little or no neutralizing power. The frog is also immune to tetanus under normal conditions. When the toxin is injected, the body temperature being too low to allow the combination between toxin and nervous tissue to take place, no tetanus results. If, however, the frog is placed in a tank of warm water and kept at the normal temperature of the human body it succumbs to the effects of the toxin. Clearly chickens and frogs are

resistant to tetanus for very different reasons. The first, because of the different chemical compositions of the brain; the second, because of physical incompatability to the parasite.

Zinsser in discussing nonheritable natural immunity, says:

> The individual differences in resistance which unquestionably exist among members of the same species and races are very difficult to explain, but, as far as we can tell anything about them at all, they seem to depend upon variation in what is popularly spoken of as "general condition."

This indicates that the counteracting chemicals are present in greater or less amounts even in organisms that readily succumb to a given disease. Or, stating it in another way, the particular tissue to be attacked can suffer injury and recover if the host is well nourished and in an optimum environment for its existence. This phase of natural immunity leads directly into the phenomenon of acquired immunity.

Injuries or wounds in plant and animal life not only stimulate repair but also growth and rejuvenescence. If the injury is sufficiently severe, or repeated often enough, the energy required to stimulate the affected part is too great a tax and the organism dies. Any factor which reduces the energy of the organism, such as fatigue, malnutrition, unfavorable temperature, etc., should reduce its resistance to disease, and this is what actually happens.

In naturally acquired immunity, the injury caused by the toxin of the invading organism stimulates an overproduction of the material used up in neutralizing it, so that subsequently more and more of the toxin can be neutralized without fatal results. Similarly in induced immunity, the injection of increasing amounts of toxin can be borne owing to the preponderantly greater amounts of neutralizing substances elaborated. In immunization work, one of the standard methods is to inject sublethal doses of fully virulent organisms in order to stimulate this great overproduction of these counteracting chemicals, which are cast into the blood and neutralize the counteracting poison at once. Such immunized animals may or may not, depending upon the particular antigen or toxin, retain this immunity for life.

Since the beginning of modern medical sceince it has been known that people having once recovered from such diseases as plague, cholera, smallpox, or yellow fever will not again during their lifetime contract it; but lasting immunity is not conferred by one attack of such diseases as pneumonia, tetanus, or influenza. In attempting to explain or make use of these phenomena a great branch of physiological chemistry has been developed.

The most comprehensive attempt to explain the causes of immunity is that offered by Ehrlich (*10*), in which he conceives the cells of host and parasite as aggregations of complex molecules which are themselves complex. Complex molecules react with one another through certain of their side chains, but only when these side chains have a certain definite correspondence in structure. The reactions of immunity represent only a repitition of the processes of normal metabolism. A receptor is an outlying part (the chain) of the cell which is able to combine, by means of a so-called haptophorous group, with foreign (protein) molecules. The haptophorous group of the receptor is able to combine with a food molecule or with a toxin molecule. Such combination stimulates the cell to produce more receptors which, in the case of toxin combination, may result in over production, the

superfluous receptors becoming detached from the cell. These are called amboceptors because they have two haptophorous groups, and in the immunizing process they act as links to bind the invading cells to the complements which are normally present and which, when so united to the foreign cells, are able to destroy them by means of a zymotoxic or toxophore group. The amboceptor with its complement constitutes a cytotoxin, hemolysin, or bacteriolysin. Other detached receptors act as antitoxins, as agglutinins, or as precipitins. Toxins have a haptophorous group by which they combine with antitoxins, and a toxophorous group to which their injurious effects are due. A toxin which has lost its toxophorous group, as by heating, is called a toxoid. A complement which has lost its zymotoxic group is a complementoid and an amboceptor that has lost one of its haptophorous groups is called an amboceptoid. The presence of a foreign cytotoxin leads to the presence of an anticytotoxin, which may act on the amboceptor or on the complement.

Whether Ehrlich's or similar theories can be extended to account for all the complex phenomena of reistance and immunity in plants and animals remains for the future to decide. Like the hypotheses in physics and chemistry, it offers a shorthand method for explaning the facts. It helps to make a clearer understanding of the phenomena of both natural and acquired resistance and immunity and also explains in a logical way the change in the virulency of organisms, in so far as there is any explanation at present. For example, as Walker (*59, p. 34*) has shown the pathogenic organisms themselves may be immunized against immune serums by cultivating them in media containing increasing portions of the immune serums.

It is not too much to suppose, therefore, that pathogenic organisms themselves are subject to contagious diseases, suffer from malnutrition, and benefit by becoming acclimated, in response to the same laws that operate in higher organisms. Changes in virulency can probably be explained as the interaction of these factors in the majority of cases; but the plasticity or heterozygous character of some forms offer the possibility of genetical change by selection. The brief time required for each generation in many of the lower forms and the large numbers produced would make this a quick and permanent method of adapting an organism to its environment. Since, in general, the lower forms are more constant or stable than the higher forms, it is probable that such genetic change by selection is very limited.

From the foregoing discussion it seems that disease-resistance phenomena in both plants and animals are due to the same general causes. The hosts of a given parasite may vary both morphologically and physiologically; and according to their physico-chemical complex react diversely in their irritability and response to such factors as temperature, moisture, light, nutrition, and poison. The same organism will react differently to the same stimuli at different stages in its development. In so far as the subject has been studied, the parasite or disease-causing organism seems to vary also, and according to the same laws that govern the host. Added to this, the host is changed within certain limits, chemically, by its food supply and other external stimuli, such as other diseases or mechanical injury. The parasite probably has its diseases and is no doubt changed chemically by them and also by its changing food supply. In the delicate balance between these two organisms, host and

parasite, there is a physico-chemcial complex so intricate that it has thus far been impossible to comprehend or to analyze it in all its detail. Heritable resistance represents a permanent upsetting of this balance in favor of the host, caused by the variation in its physico-chemical complex.

INHERITANCE OF RESISTANCE

Acquired resistance and induced immunity, like other acquired characters, have not been shown experimentally to be inherited in the ordinary sense of the term, but each generation must recover from a given disease or be vaccinated (or inoculated with the proper virus) in order to acquire immunity. Inherent or natural resistance is definitely passed on from generation to generation like morphological characters. When such a resistant race is crossed on a susceptible one, resistant and susceptible races may be isolated in subsequent generations, which show that the same laws of inheritance which control other characteristics are in operation in determining the causes of resistance. Biffn (*2*), in England, found resistance to yellow rust *Puccinia glumarum* Erikss. and Henn. and resistance to mildew *Erysiphe graminis* DC. in barley to be a simple mendelian recessive. One of his resistant hybrid wheats has become of commercial importance and has retained its resistance at Cambridge for 16 generations. Nilsson-Ehle (*34*), in Sweden, corroborated Biffen's findings as to the heritability of resistance to yellow rust, but in his crosses he obtained segregates more resistant and more susceptible than the parents, which he interpreted as being due to modifying multiple factors. Hayes, Parker, and Kurtzweil (*15*), in America, found the same phenomenon in connection with resistance to stemrust *Puccinia graminis* Pers. in wheat and in addition found linkage between resistance and certain durum and emmer-like characters.

Other genera that have been studied genetically in regard to resistance to various diseases, in general show the same type of inheritance. McRostie (*30*) ,working with two independent strains of the bean anthracnose organism *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., showed resistance to anthracnose to be dominant to susceptibility. When a bean resistant to both strains was crossed on one susceptible to both, a ratio of 9 resistant to 7 susceptible was obtained in the F_2 generation. Either strain alone gave the expected 3 to 1 ratio. Later (*31*) he gave additional data including 36 F_3 families which supported his earlier interpretation. In addition he reported rootrot (caused by *Fusarium martii phaseoli* Burk.,) and mosaic resistance in beans to be partially recessive. He interpreted his F_2 results according to the ratio 9 susceptible to 7 resistant, which was supported by the performance of 183 F_3 families for rootrot and 329 F_3 families for mosaic. Parker (*39*) published a preliminary report on the crownrust of oats, *Puccinia lolii avenae* McAlpine, in which he concludes that resistance is recessive but was caused by multiple factors. Orton (*36*, *37*) described the transference of wilt resistance of the stock melon to the watermelon by crossing, and similarly, resistance to wilt and rootrot was transferred from the Iron cowpea to one of the Whippoorwill type.

Among the farm crop plants that have attained commercial importance through their disease-resisting qualities, but which originated by selection or introduction, may be mentioned the following:

1. Upland and Sea Island cotton resistant to wilt. (Webber.)
2. Potatoes resistant to late blight and scab. (Jones and Stuart.)

3. Flax resistant to wilt. (Bolley.)
4. Cantaloup resistant to leafblight. (Blinn.)
5. Clover resistant to anthracnose. (Bain and Essary.)
6. Durum wheat in the Dakotas (Carleton) and Kanred wheat in Kansas (Roberts) resistant to rust.
7. Tobacco resistant to rootrot. (Johnson and Milton.)
8. Grape resistant to Phylloxera. (Viola and Ravaz.)

A genetic analysis of the resistance of these crops after crossing them with susceptible races would be required to show the type of inheritance in each case. Such investigation would be more concise and the interpretation clearer if a standard method of inoculation were adopted and a quantitative rather than a qualitative measure of resistance were used, as is being done in rust resistant work with cereals (*38*). The analysis of the inheritance of rootrot in tobacco (*21*) should prove especially interesting because of the ease with which crosses are made and also on account of the amount of genetic work already done on tobacco. It is difficult to interpret much of the past work because inoculation was left to chance or natural agencies, which allowed part of the individuals to escape infection and the resulting segregates were arbitrarily classified as resistant or susceptible, when as a matter of fact every graduation from complete susceptibility to complete immunity occurred. In some cases (*35*) different stages in the life history of the organism apparently do not have the same infective power.

Whether resistance of a given host against a given parasite will be maintained indefinitely in a given environment remains for the future to decide. Resistance in certain crops (*46*) may be only temporary. The resistance of the host might be apparently lost through the introduction of a new physiological race of the parasite to which it was susceptible. An example of such an occurrence is recorded by Levine and Stakman (*25*). They obtained a strain of stemrust from Oklahoma that could attack Kanred wheat although Kanred continued to be very resistant to the two common races of stemrust in Kansas. According to Evans (*11*) Bobs Rust Proof wheat was resistant to stemrust at Pretoria, South Africa, but was badly attacked in the Low Country of the Transvaal. This may have been due to the change in environment or to the presence of a different biologic form of the fungus encountered in the Low Country. Evans also reported an extraordinary case in which the pathogenic properties of stemrust on wheat were increased nearly five fold by passing one generation of its existence on the F_1 generation of a cross between a resistant and a susceptible variety (Bobs $\times$ Wol Koran). Bobs normally produced one-fifth as many pustules as Wol Koran, but when infected with spores from the F_1 generation it produced one-third as many. In this case the F_1 generation had apparently acted as a "bridge" to permanently increase the virulency of the rust and had also decreased the difference between the resistant and susceptible varieties. If this should prove to be a general rule, breeding for resistance would not only be without avail but would actually be a very dangerous practice on account of the danger of developing and spreading super-virulent cultures of the parasite. It is to be regretted that Evans did not grow an F_2 generation of the host to see whether super-resistant segregates could be obtained. He worked with comparatively few plants in the greenhouse, and his results may have been due to some uncontrolled factor, for other

investigators have not been able to duplicate his results. Biffen (*3*) is positive that such "bridging" or increase in virulence did not occur among any of his many hybrids in England. Stakman and his associates (*45–53*) in America, after long and careful work with stemrust on its many hosts, have failed to find any indication of "bridging" or increased virulence of the parasite. They grew it on hybrids of resistant × susceptible wheat varieties from the F_1, F_2, and F_9 generations. They used different physiological races of the parasite and many species, including several genera of host material. In no case did they find an intermediary host that would increase the virulency of the rust. On the other hand, they found that the parasite became sickly when cultivated on a resistant host and often could not be maintained more than a few generations.

According to Vavilov (*58*) "bridging species" are found in *Puccinia Symphyti Bromorum* F. Müll. (M. Ward and Freeman), *Erysiphe graminis* DC. living on Bromus (E. Salmon), in *P. graminis forma* sp. *tritici* (Freeman and Johnson), and *Sphaerotheca Humuli* on Alchemilla (Steiner). It will be noted that these few cases deal with relatively weakley specialized fungi, when compared with the smuts, among which biologic races of different infecting power are known to exist; and the hosts used contain races varying from complete susceptibility to immunity. The conflicting accounts of American investigators may be explained on similar grounds. For example, Johnson (*20*) was able to transfer timothy rust *Puccinia phleipratensis* Erikss. and Henn. to barley by means of oats as a bridging host when it could not be transferred from timothy direct, but Stakman and Jensen (*47*) transferred timothy rust successfully to oats, barley, rye, wild oats, oat grass, orchard grass, wild rye, rye grass, and rough brome, without the use of a bridging host.

The biologic races of hosts and parasites, differing in resistance and virulency and the difficulty of keeping pedigreed cultures of each not only make accurate work difficult but make it almost impossible for two investigators working in different parts of the country to corroborate each other's work. "Wheat" and "stemrust" are terms not sufficiently specific for work of this kind. The particular variety of wheat and the biologic race of the rust must be known.

Plants vary also in their resistance to the attacks of insects. Torssell (*56*), in Sweden, found certain winter wheats that were not harmed by the six-spotted leafhopper while other varieties in the same field were destroyed. In 1916 in the variety test of field peas planted at the Washington Agricultural Experiment Station, the seed of Alaska peas was infested with pea weevil. At harvest time the Alaskas contained more infested than good seed, while the Bangalias growing beside them contained only a trace. The adult weevil is winged but evidently preferred the Alaskas. It is a common observation among potato growers that the Colorado potato beetle will destroy certain varieties before attacking others. For example, if Early Rose and Rural New Yorkers are planted in alternate rows in a garden, the beetles will strip the leaves of the Early Rose before harming to any appreciable extent the Rurals. But perhaps the most striking example of resistance to insect attack is that of certain grapes resistant to the plant louse *Phylloxera vitifoliae* Fitch. described by Bioletti (*4*). When resistant and susceptible varieties are crossed, resistance seems to be dominant. Such hybrids have become the foundation stock for grafting with the choice wine, table, and raisin grapes of Europe. Phylloxera feeds on the

roots of the grape and produces galls or "tuberosites" which, on the susceptible varieties, enlarge enormously, and in two or three years kill the vines. This growth is caused by a poison which is injected by the insect. The resistant varieties do not react to this poison, and in addition the insect does not multiply so rapidly on it, which indicates a repellant effect of the juice of the resistant plant on the life of the Phylloxera. Between two and three million acres of vineyards in France and California have been destroyed by Phylloxera during the past 50 years and have been replanted to choice varieties that have been grafted on to resistant stocks.

Little work has been done on the inheritance of disease resistance in animals. The nearest approach to an exhaustive critical investigation in this subject, is that by Little and Tyzzer (*26*). They could transplant the carcinoma tumor designated as *J. w. A.* at will on the Japanese waltzing mouse, but the common mouse was immune to it. The F_1 generation of these two races was very susceptible, there being but 1 in 62 that failed to react. The F_2 and F_3 generations were very nearly immune, there being but 3 reacting positively out of 221. The F_1 generation, back-crossed with the susceptible parent gave mostly susceptible offspring (64 positive to 4 negative), but when back-crossed with the immune parent only immune offspring were produced (112 negative reactions). Altogether 629 mice were used in the investigation. The authors interpreted susceptibility to transplanted carcinoma tumor *J. w. A.* as being due to a complex of dominant multiple factors but considered their data insufficient to arrive at an approximation of the number involved.

From the illustrations given above it will be seen that the researches already made on the inheritance of disease resistance indicate that, in the main, whether due to physical or chemical causes, Mendelian laws of segregation and recombination control resistance phenomena generally. Artificial crossing and selection of large numbers should help clear up the difficulties in making practical use of these laws in controlling crop and animal pests.

BUNT RESISTANCE PHENOMENA IN WHEAT

THE PARASITE

There are two species of bunt, *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. *T. tritici* is the most common and is the one generally referred to in the literature, and, unless otherwise designated, is the species referred to throughout this paper.

The smuts (order Ustilaginales) were among the first recognized plagues of plants. Pliny (*41*) and Theophrastus (*54*) wrote about smut outbreaks in grains and considered them due to untoward weather and soil conditions which caused a morbid transformation, that is, putrifaction, of the tissues of the grain. In 1801 Persoon (*40*, p. 224) described stinking-smut as being due to a fungus. Meyen (*33*, p. 108–119) as late as 1841 wrote that the smut powder in a dissolved condition entered the roots and caused the sap to engender the same disease. But Kühn (*23*) was the first to prove that the fungus from the germinating spore actually enters the young wheat plant, thereby starting infection that results in the smutted head.

According to Arthur (*1*) farmers reported a bunt outbreak in 1859 in the Haw Patch district of Indiana that took half the crop. Although such epidemics are not common in Indiana, another one occurred in this same district in 1887 and in some fields caused an almost total loss.

In total harm done in reducing the world's wheat crop, bunt is second only to rust (*19*). In the wheat section of the Pacific coast in America in 1919 it is estimated that between two and three million bushels were lost on account of it. Besides the actual reduction in yield, the spores clinging to the grain and straw may be harmful to certain animals, and added expense is required to clean the grain for flour if the crop is even slightly contaminated. It is known and dreaded in all countries where wheat is grown. The countless spores in the separator during thrashing are sometimes exploded by the static electricity of the revolving cylinder and a fire is started that destroys both machinery and crop (Pl. 1, A). During thrashing the liberated spores are disseminated by the wind, and, as Heald and George (*16*) have shown, distributed at the rate of more than 35,000 spores to the square inch during a single season over distant fields about to be planted to fall wheat. Under such conditions seed treatment will not prevent a smutty crop of winter wheat the following year if the season is favorable for infection by the wind-borne spores. Experiments have shown that in a few weeks after the first rains, however, the spores germinate, and in the absence of wheat seedlings to infect, the fungus dies within a month or 6 weeks. Spores have been kept in the dry for 12 years which would still germinate (Woolman and Humphrey.[3])

McAlpine (*28*, *29*) describes the spores of *Tilletia tritici* (Bjerk.) Wint. as globose with reticulate roughened surface 15 to 22 μ (average 16μ) in diameter. Under suitable temperature and moisture conditions the resting spores germinate in air or water. In water a long septate, unbranched mycelium is produced, whereas in air a short, septate, promycelium is produced, at the end of which are borne 3 to 10 filamentous or sickle-shaped conidia. These conidia, fusing in pairs, produce the infection threads by means of which the parasite enters the host. This is accomplished between germination and the beginning of photosynthesis of the young wheat plant. Once established, the fungus grows until fruiting time, keeping pace with the growing point without serious injury to the host. It then produces a mass of hyphae in the walls of the young ovules, which results in such a quantity of odoriferous spores as to fill the ovaries to the point of bursting—that is, making "smut balls" about the size of wheat kernels.

The resting spores are never produced except in the ovaries of the host. The parasite remains in a vegetative condition during the lifetime of the host—whether it be 2 months or 10. The physico-chemical changes that take place in the wheat plant as it approaches maturity stimulate the bunt organism to similar changes, for it quickly spreads its hyphae through the remains of the nucellus and deposits masses of its own fruiting spores in the space normally occupied by the endosperm and embryo of the wheat.

Bunt, in common with other smuts, apparently consists of but a single biologic race. Like other disease-producing organisms, it is selective for certain tissues (*24*). The nodes and growing points of the host are the

[3] Woolman, Horace M., and Humphrey, Harry B. Studies in the physiology and control of bunt or stinking smut of wheat. U. S. Dept. Agr. Bul. (In press.)

tissues in which it is most frequently found in this case, and the fungus no doubt produces toxins which bring about the deformity characteristic of a smutted plant. (Pl. 1, B; 2, A.)

According to Woolman,[4] bunt infection takes place during the early seedling stages, through the walls of the coleoptile. He is unable to find any trace of mycelium except in the coleoptile until the seedlings are about 8 days old. By the time they are 15 days old the mycelium has penetrated to the inner side of the second leaf sheath. As the plant grows older the mycelium penetrates farther and is carried upward mechanically with the growing point, living filaments being found most frequently at the nodes and in or near the actively growing parts. The older parts of the mycelium disappear, apparently being digested and absorbed. He finds that the so-called immune varieties are readily infected and that resistant and susceptible seedlings show no difference during the first two weeks. By the time they are a month old, however, the mycelium in the resistant varieties is more restricted in its ramifications and appears lacking in stainable cell contents. The "resistance" is evidently chemical in nature and begins to restrict the growth of the mycelium shortly after the genesis of photosynthesis. He rarely finds evidence of infection at more than one point, although he found one head containing spores of both *Tillitia tritici* and *T. levis*, which proves that two infections did take place, each functioning at maturity.

Heald (*17*) believes that multiple infection is required to develop a smutted plant. He found that Marquis wheat, which is very resistant to bunt, could endure from 500 to 1,000 spores per grain without producing bunted heads in the succeeding crop. When seed carrying 100,000 or more spores per grain were planted half the resulting crop consisted of bunted heads. Jenkins Club, which is very susceptible, produced a trace of bunt with as few as 100 spores per seed. When the inoculation reached 40,000 or more the resulting crop produced from 80 to 100 per cent of bunted heads.

If the multiple infection hypothesis be the correct explanation of this phenomenon, it might be compared to induced immunity in animals brought about by sublethal injections of fully virulent organisms. Infection in a great number of different places—that is, a lethal dose—would use up the "antitoxin" already present in the plant so rapidly that the pathogenic relationship could be established. The resistant varieties would either contain more of the "antitoxin" or a greater capacity for developing such material. Much more work will be required to establish this point. The data as published (*17*) might be interpreted as increasing the chances for infection or producing the external conditions that would make possible the penetration of the host cuticle (*7*). It has been shown that the amount of bunt is greatest in wheat planted for September 15 and only slightly less on October 1, at Pullman, Wash., the amount growing less and less in plantings either earlier or later. In view of this fact, as well as the fact that varieties differ in relative susceptibility, makes the proposition of determining by a spore count whether a given lot of seed needs treating, a difficult and precarious one, for winter wheat. A general prediction of the amount of smut to be expected in spring wheat would be less hazardous.

[4] My former colleague, Mr H. M. Woolman, has done much cytological work on the relationships of bunt and wheat, and he has kindly permitted me to make the above statements of his unpublished work.

Summarizing the evidence at hand, bunt appears to be a highly specialized parasite, consisting of but a single biologic race and existing on but a single host genus, Triticum.[5] Its spores are disseminated both on the seed of the host and by wind to fields of fall wheat. Infection takes place only during the seedling stage of the host and to be effective may require multiple infection. It produces but one crop of spores a year, these being deposited in the wheat ovaries following the flowering period. Next to rust, it is the most destructive parasite of wheat. Besides the actual loss due to bunted heads, additional losses occur through feeding smutty grain to animals, through lowering the vitality of the seed by treatment with disinfectants, through the time and expense of treating the seed, through fires caused by explosions of spores during thrashing, through extra cleaning required in milling, and through experiments of farmers, such as planting out of season, changing seed, and trying other unadapted plants in an effort to avoid a smutty crop.

THE HOST

There are eight commonly recognized species of wheat in American agricultural literature. According to Tschermak (*57*) Einkorn, *Triticum monococcum* is quite distinct from the others and generally will not cross with them, although Blaringhem (*5*) succeeded in crossing it with durum and Polish wheat. Emmer, *T. dicoccum* Schr., is thought to be the progenitor of the other six. Durum, *T. durum* Desf., Polish, *T. polonicum* L., and Poulard, *T. turgidum* L. are inter-fertile but show about 50 per cent sterility when crossed with the other three. Common wheat, *T. vulgare* Vill., Club wheat, *T. compactum* Host., and Spelt, *T. spelta* L. are all fertile inter se.

Triticum generally shows intersterility with other genera, although crosses have been made with Secale (rye), but the F_1 generation is nearly always sterile, rarely producing seed. Love and Craig (*27*) reviewed former work on wheat-rye hydrids and reported a cross between Dawson Golden Chaff and common rye in which they obtained a single plant in the F_1, F_2, and F_3 generation, there being but one seed produced in each of the first two generations. The F_3 generation was not so nearly sterile, for it produced a number of seeds from which a variable population of F_4 segregates were obtained.

In common with many other members of the grass family the bread wheats have hollow stems closed at the nodes and two-ranked parallel-veined leaves consisting of sheath and blade. The sheath envelopes the stem with the edges overlapping. The flowers are perfect, arranged 1 to 6 on a spikelet, and the spikelets alternate on the rachis to form a spike. Two empty glumes inclose each spikelet. Each flower is inclosed in a floral glume on the outside and a palea on the inside and consists of three stamens with slender filaments and a 1-celled ovary beneath two styles with plumose stigmas. Wheat generally is self-fertilized, but natural hybrids sometimes occur in arid climates.

After germination the plant passes through a vegetative stage in which it produces a cluster of leaves (the stool), tillering from a zone of active

[5] Since completing this manuscript I found two smutted ryelike plants in an F_2 generation of Hybrid 128 wheat Rosen rye. The characteristic odor and reticulated surfaces of the spores make it almost certain that the casual organism was *Tilletia tritici*, (Bjerk.), Wint. The same season (1921) F. J. Stevenson found two heads of what looked like bunt on common rye in our cereal nursery at Pullman, Wash., near the place where the two rye-wheat hybrid plants had been found.

tissue near the surface of the earth. With the approach of summer the stems elongate rapidly, the seeds develop, and the plant dies. The vegetative stage is completed in from one to eight months, depending upon temperature and variety.

The vegetative period exhibits some interesting variations. A fundamental varietal agronomic difference of great practical importance, which is entirely ignored in most taxonomic nomenclature, is the habit of growth during this vegetative period that distinguishes winter and spring wheats. A typical winter wheat, in the north temperate zone, will not head out or produce grain if planted in the spring. It remains as a green grass clump all summer and dies the following winter. If it is pastured or the leaves frequently cut off during the summer, it may live through the winter and the following summer head out and produce a normal crop of grain. Spring wheat, planted in the spring, passes through the vegetative stage in a few weeks and then the culms rapidly elognate, the head develops, and a normal crop is produced. If both are planted in the fall, the winter wheat lives through the freezing and thawing of a severe winter and produces a normal crop the following harvest under conditions in which the spring variety would winterkill. The time required from flowering to the maturing of the grain varies greatly with the changing climatic conditions. High temperatures, bright sunlight, and strong winds favor rapid maturation. Cool, cloudy weather with little wind favors the maximum length of time between flowering and maturity. The elongation of the stems, flowering, and fruiting are completed in from 30 to 60 days or longer, depending upon weather conditions.

Most of the cultivated wheats are of the species *Triticum vulgare*, but in certain districts the durum and club wheats predominate. Durum is produced in the Dakotas and western Minnesota principally, and the club area is confined, for the most part, to the Pacific Northwest.

Many investigators have classified comparative susceptibility to disease according to genera and species. (Jaczewski (*18*), Carleton (*8*), Freeman and Johnson (*12*), Stakman (*52*), Kirchner (*22*), and Reed (*42*).) For wheat they have shown that einkorn is generally resistant to all rusts and mildews. The durums, polish, poulard, and some of the emmers are partially resistant, but the common and club wheats, the spelts and part of the emmers are generally classified as susceptible. This shows a remarkable parallelism with the sterility groups and suggests the probability that the physical and chemical factor differences that cause sterility are also responsible for the differences in resistance. It would be expected, if this were the case, that specific sterility factors would be linked with resistance, and both would be linked with the morphological differences that make up the species. Hayes, Parker, and Kurtzweil (*15*) actually found such linkage, together with the expected sterility, in crosses of common with both durum and emmer. Linkage was not complete, however, for out of a large number they obtained a very few segregates of common type that were resistant to stemrust and some of emmer and durum type that were susceptible. From this it appears that the physico-chemical factor complex responsible for sterility in crosses of so-called immune and susceptible races is not identical with those producing resistance, although very closely associated. In fact, certain sterility factors may be the identical factors that cause certain kinds of resistance, for resistance is specific for each parasite. A wheat may be susceptible to stemrust and resistant to yellow rust, or resistant to mildew and susceptible to both rusts (*2*, *9*).

Bunt, like the rusts, finds certain species more congenial hosts than others. Table I, compiled from the tests of 1919 and 1920 at the Washington Agricultural Experiment Station, Pullman, Wash., shows the comparative resistance of the eight species as tested.

TABLE 1.—*Comparative bunt resistance of the eight species of wheat*

Species.	Number of tests.[a]	Total number of plants counted.	Percentage of bunt produced.
Einkorn (*T. monococcum*)	4	87	0.0
Polish (*T. polonicum*)	3	66	7.7
Emmer (*T. dicoccum*)	10	155	9.6
Spelt (*T. spelta*)	21	515	9.7
Durum (*T. durum*)	40	1,256	29.3
Poulard (*T. turgidum*)	9	264	33.5
Club (*T. compactum*)	98	4,296	64.1
Common (*T. vulgare*)	666	25,009	70.2

[a] The actual number of distinct races or sorts was but slightly over 500 in round numbers. Many of the 1919 plantings were duplicated in 1920.

Taking the average percentage of bunt produced as the index of resistance, the eight species may be arranged into four groups. Einkorn is immune, polish, emmer, and spelt are very resistant, durum and poulard are intermediate, and club and common are very susceptible. It would require a much more exhaustive test to establish these findings as general laws for specific bunt resistance, but there are two deductions that may be made. 1. Species of Triticum differ in their susceptibility to bunt and in a manner analogous to specific rust resistance and also analogous to their genetic relationships. 2. Spelt forms a notable exception, falling into the very resistant class instead of the susceptible class that its genetic and rust-resistance relationships call for. These relationships are established by the work of several investigators (*15*, *57*, *58*), and the different behavior of spelt in respect to bunt resistance is established in Table 1 by the performance of 21 rows and more than 500 plants.

It has been found that the common and club wheats vary within rather wide limits among the different races of the same species, a few selections of both being very resistant, more being intermediate, but the majority being very susceptible.

Resistance to some diseases seems to break down when the host is grown in another environment. The information available on bunt resistance indicates that a wheat resistant to bunt will remain so in all climates. For example, Turkey (Washington, No. 326) is resistant under conditions favoring maximum infection at the experiment stations of Kansas, Minnesota, California, Oregon, Idaho, and Washington.

A comparison of a large number of varieties, known to be pure lines, in two districts of distinctly different climate should give a clear idea of the probable constancy of bunt resistance in different environments. This can best be done by means of a correlation table in which the comparative resistance of each variety in one locality is measured in relation to its comparative resistance in the other locality. If there is perfect agreement, the coefficient of correlation is 1. If they vary independently in each locality, without regard to the comparative resistance

in the other, the coefficient of correlation equals 0. Table II presents such a correlation.

TABLE II.—*Correlation of 150 varieties of wheat in respect to bunt resistance* [1]

Class centers.	5	15	25	35	45	55	65	75	85	95	Pullman frequencies.
5	4	....	1	....	1	....	....	....	....	....	6
15	1	1	....	1	....	....	....	....	....	....	3
25	1	....	2	....	....	....	1	....	2	....	6
35	....	1	....	....	2	....	....	3	....	....	6
45	....	....	2	3	....	1	2	1	1	....	10
55	....	1	....	....	1	2	1	1	....	2	8
65	....	....	1	2	2	5	2	2	4	4	22
75	....	1	....	2	1	2	....	4	5	1	16
85	1	....	1	....	1	1	3	4	6	5	22
95	....	....	....	1	1	....	1	6	12	30	51
Moro frequencies	7	4	7	9	9	11	10	21	30	42	150

[1] Percentage of bunt at Moro, subject; percentage of bunt at Pullman, relative; 1919.
Coefficient of correlation $= 0.654 \pm 0.0315$.

A comparison of 150 selections of wheat (mostly *Triticum vulgare*, a few being club and durum) tested at Pullman, Wash., and Moro, Oreg., show a correlation coefficient, according to Table II, of 0.654 ± 0.0315. Moro has an arid climate with an average rainfall of 11.6 inches. The elevation is 1,800 feet; the soil, a fine silt loam. Pullman has an annual rainfall of 21 inches, a clay loam soil, an elevation of 2,500 feet, and a lower summer temperature than Moro. The Moro data were obtained by counting the heads of bunt and wheat from which the percentages were figured. The Pullman figures were obtained by the combination plant and head count described on page 460. Taking into account the differences in climate and methods, the high correlation is significant. It shows that resistance and susceptibility are fundamental differences and are not easily changed by environmental conditions. A large number of these varieties have been tested at Davis, Calif., Aberdeen, Idaho, and Corvallis, Oreg., under wide differences of soil and climate, and the indications are that the common varieties that are decidedly resistant or susceptible are outstanding in those respects at all places where tested. Table II shows only four varieties less than 10 per cent bunt at both Stations, but 30 varieties more than 90 per cent susceptible. The average of all varieties at both Stations is 70 per cent, which is very near the figure given for the *T. vulgare* group in Table I, in which a much larger number of varieties is represented, covering the two-year test (1919 and 1920) at Pullman.

It is very seldom that a row of wheat, containing, say, from 50 to 100 plants produces nothing but bunt heads, no matter how susceptible the variety is or how favorable the conditions for infection are. A small number of wheat heads, and usually a few plants, escape the fungus and produce normal seed. It might be assumed that these occasional plants were resistant mutants. To test this possibility 10 bunt-free plants of hybrid 143 were selected in 1913, and for three years smut-free plants from the most resistant row were selected. At the end of the third year the selected plants produced 85 per cent of bunt as compared with 80

per cent for the unselected check. Other tests of selections from Turkey and Red Russian add proof to the conclusion that the bunt-free plants in varieties planted under conditions favoring maximum infection are not resistant mutants but rather escape infection by accident.

BREEDING FOR BUNT RESISTANCE

METHODS

In testing different wheats for comparative resistance to bunt, conditions favoring maximum infection were, as nearly as possible, maintained. The seed was inoculated with fresh viable spores just before planting. This was accomplished by stirring into each packet of seeds from 1 to 5 per cent of its weight in bunt spores, so that each seed was literally blackened by spores clinging to it, besides a surplus of loose spores among the seeds. The inoculum was obtained from the smutted heads of many different varieties of wheat in the field, in order to get a representative sample of the organism in its native environment. The heads were ground up and the spores sifted out and kept in a cool, dry place during the planting season. The packets of inoculated seeds were planted by hand in rows 18 inches apart. The seeds were spaced 4 to 6 inches apart in the row to avoid confusion in separating the plants at harvest time. Each experiment, whether a variety test or an F_3 family, was planted at approximately the same date to avoid error due to changing seasonal conditions. As many as 10 men were required at times in order to plant a given experiment in a single day.

The field was kept free of weeds and volunteer grain by rotation and cultivation, corn and field peas preceding the cereal nursery in a 3-year rotation in every case. The rainless summers of eastern Washington make clean cultivation under these conditions comparatively easy. A stake bearing a printed label with date, name of experiment, name of variety and pedigree number was placed at the end of each row. The records at harvest time were obtained as follows: To get a quantitative measure of resistance, the plants of each row were pulled and separated into three piles (bunt-free, all bunt, and part bunt); and the number of plants in each was recorded in a field note book. The partly bunted plants were then divided into heads of wheat and heads of bunt, and the numbers were entered in their proper columns. With these five figures it is easy to reduce the amount of bunt in a given row to terms of a single number for direct comparison with the amount produced by any other row. The computation used gives the total bunt in terms of percentage of the whole row, according to the formula $ab+c=d$, in which a is the percentage of bunted heads on the partly bunted plants, b is the percentage of partly bunted plants in the row, c is the percentage of entirely bunted plants in the row, and d is the total percentage of the row that is bunted. The computations were made with a calculating machine and checked with a slide rule. The results thus obtained are not materially different from those obtainable by a straight head count of all the heads in the row. This method takes much less time and gives information that a straight head count would not give—that is, the quantity of partly bunted plants and the amount of wheat produced on them, which in itself is an important measure of resistance, and is useful in checking errors in counting or computations.

The work was all carried on out of doors, in the field, the main care being to have uniform conditions for all the rows of a given test, so that

the differences in bunt resistance would be attributable directly to differences in the constitutional make-up of the wheats compared.

PARENT STOCK

Of the eight varieties to be discussed in relation to their bunt resistance, six are of wide commercial importance. Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones Winter Fife are the five most important winter wheats in the State of Washington and are named in the order of their importance from the standpoint of total production. They represent more than three-fourths of the winter wheat produced in the Northwest. Alaska is not commercially grown except in isolated sections where an occasional farmer has been the victim of propagandists who have made extravagant claims for it as a wonderfully productive and hardy wheat. Of the two spring varieties, Marquis is third in production in Washington and is universally grown in the Great Plains area of the United States and in northwestern Canada. Florence is of some importance in Australia but has not been grown in America except for experimental purposes.

Taxonomically all are *Triticum vulgare* except Hybrid 128, which belongs to *T. compactum* and Alaska which is a poulard, *T. turgidum*. Turkey, Florence, and Alaska are resistant (Pl. 2, B) and Hybrid 128 and Jones Winter Fife are susceptible to bunt under all conditions. Fortyfold and Red Russian are intermediate but approaching the susceptible varieties (Pl. 3, A). Marquis is resistant when sown in the spring but intermediate or susceptible when fall-sown. Table III gives the amount of bunt produced in the tests from 1915 to 1920. The season of 1917 was very unfavorable, and the wheat did not come up till spring. Since none of the varieties produced any appreciable amount of bunt, the record of that year is omitted.

TABLE III.—*Annual percentage of bunt produced on parent stock for the years 1915 to 1920, with the 3-year average for 1918 to 1920, and the total number of plants in the tests during the latter period*

Variety.	Percentage of bunt.						Total number of plants in 3 years 1918–1920.
	1915	1916	1918	1919	1920	Average 1918–1920.	
Turkey	4.6	1.7	0.6	2.4	7.6	3.5	2,883
Marquis [a]	.....	.....	3.4	7.3	3.5	4.7	813
Florence	.....	.....	.9	8.1	12.2	7.1	880
Alaska	.....	.....	.0	7.1	20.1	9.1	312
Red Russian	67.8	85.6	49.9	77.4	56.1	61.1	2,118
Fortyfold	73.5	71.2	49.6	76.4	65.2	63.7	479
Hybrid 128	86.6	96.4	63.0	98.1	77.6	79.6	685
Jones Winter Fife	89.3	87.4	78.3	94.2	73.6	82.0	317
Average first 4 varieties	.....	.....	1.2	6.2	10.8	4.5	.....
Average last 4 varieties	79.3	85.2	60.2	86.5	68.1	75.9	.....
Average of both resistant and susceptible [b]	.....	.....	30.7	46.4	39.5	38.9	.....

[a] Marquis was spring-sown. All the others were sown in the fall throughout the tests.
[b] See Plates 2, B, and 3, A, for the type of heads of these eight parent varieties.

There is considerable variation from year to year, but the varieties generally maintain their relative positions. The four resistant ones are always comparatively resistant and the susceptible ones are always comparatively susceptible, regardless of seasonal fluctuations. Turkey, representing the extreme resistant type, and Fife, representing the extreme susceptible type, show less variation than the others. Marquis shows less variation than any of the others during the three years recorded and is so uniformly resistant that farmers often take advantage of it and do not treat the seed before planting. It was a matter of considerable surprise, therefore, when it was discovered that Marquis was susceptible when planted in the fall. In 1919 a fall sown row of inoculated seed produced 661 heads, 489, or 74 per cent, of which were bunted. The same phenomenon was observed in 1920, the 14 plants that survived the winter producing 31 per cent bunt, compared with 3.5 per cent produced from a spring planting of 90 plants in the same field. The record of Florence, the other spring wheat, is taken from fall sowings but is somewhat more resistant when spring-sown. Evidently the resistance of Marquis is different from that of the others, inasmuch as it is neutralized by the lowered temperature or the winter rest period. There seems to be no peculiar habit of growth or taxonomic difference associated with this loss in resistance, but it is a constantly recurrent phenomenon obtaining wherever tested. An example of the uniformity of these reactions is shown in the records from Davis, Calif. Seed of the three varieties Turkey, Florence, and Marquis was sent to that Station to be tested for bunt resistance in the season of 1920. Plantings of each were made November 4 and December 21, 1919. More than a thousand heads of each were produced in 1920, of which Marquis contained 37 per cent of bunt heads, Turkey and Florence containing but 7 and 6 per cent, respectively. The spring sowing of Marquis was uniformly resistant.

This does not mean that all the others have the same kind of resistance. In fact they have not, according to the segregation tests of their hybrid progeny, as will be shown later. It seems probable, from a study of Table III, that the different wheats do not react the same to climatic influences from year to year. For example, in 1920 the resistant varieties (with the exception of Marquis) produced more than twice as much bunt as their 3-year average shows, while the four susceptible varieties produced an average of 7.8 per cent less in a similar comparison. The reverse condition obtained in 1916, while in 1918 all varieties produced less than normal, and in 1919 all but Turkey and Alaska produced more than normal. It is very evident that a "good" or "bad" smut year does not apply equally to all varieties.

The percentage of bunt produced on the partly bunted plants was much less on the resistant than on the susceptible varieties, the ratio being 25 to 67 when the four resistant varieties of Table III are compared with the four susceptible ones. This may be taken as an argument in favor of the physico-chemical nature of resistance, for here there can be no question about the plants being infected. The difference is plainly one of greater incompatibility of the resistant hosts. The ratio of total bunt 4.5 to 75.9, however, shows a much stronger contrast and is the just and proper one to use as a quantitative measure of resistance or susceptibility.

SEGREGATION OF HYBRIDS

Only a few of the possible combinations of the eight parent types have been made, but the data obtained are sufficient to show the general type of the inheritance to be expected, and the crosses described below have been tested in sufficient quantities and over a long enough period of time to warrant the conclusion that resistance is definitely heritable according to the commonly recognized laws of genetics. Three types of crosses have been made: resistant × resistant, resistant × susceptible, and susceptible × susceptible. Because a smutted plant is automatically eliminated from further testing, as it produces no progeny, the seed for the F_1 and F_2 generations were not inoculated with spores but were treated with formaldehyde instead, so that both susceptible and resistant segregates were saved for the F_3 generation, which was thus the real beginning of the test. F_1 sibs had been saved, however, so that F_2 sib rows could be tested with the F_3's the same season, under the same conditions. Thus the parent varieties, the F_2's and the F_3's were all tested at the same time in the five crosses that have been carried on in sufficient numbers, and under such conditions as to make the results significant. The amount of bunt produced per row in the segregates of the F_3 generations has been arbitrarily put into 10 groups, each class having an amplitude of 10 per cent. The numbers in each class of 10 crosses is given in Table IV.

TABLE IV.—*Number of F_3 rows falling into each class, when the percentage of bunt produced from 0 to 100 is divided into 10 equal parts*

Variety.[a]	Number of rows falling into class with average percentage of bunt—										Total number of rows.
	5	15	25	35	45	55	65	75	85	95	
A × T	6	1	0	3	0	0	1	1	1	1	14
T × F	101	19	18	12	6	6	3	2	1	0	168
T × H	15	13	9	23	28	33	36	25	11	1	194
T × R	17	9	6	1	4	2	1	2	0	0	42
Fd × T	41	64	37	63	97	104	85	59	38	5	[b] 593
A × J	6	0	0	2	2	0	1	4	9	0	24
Fd × R	1	17	34	55	40	27	5	2	0	0	181
H × M	0	0	0	0	0	0	0	5	53	276	334
A × R	1	0	2	1	0	1	1	2	1	2	11
A × Fd	1	0	1	3	2	1	6	5	1	1	21

[a] T = Turkey, A = Alaska, F = Florence, H = Hybrid 128, Fd = Fortyfold, J = Jones Winter Fife, M = Marquis, R = Red Russian.

[b] From 42 plants selected at random from an F_3 population, which makes these figures really F_4's in which there has been no selective elimination of susceptible segregates in earlier generations.

The average number of plants per row varied in the different crosses from 12.6 to 60.8, and more than 60,000 plants were counted in determining the bunt percentages of the 1,582 rows listed in Table IV. The fact that the tests were made during four different years makes comparison difficult, in one way; yet it tends to add weight to the general conclusions regarding the inheritance of resistance, because of the very fact that the different seasons are included. All of the rows in which Alaska was one of the parents were grown in 1916. The Turkey crosses (except with Alaska and Fortyfold) were tested in 1918. Hybrid 128 × Marquis was tested in 1919, and the other two, Fortyfold × Turkey and

Fortyfold × Red Russian, were tested in 1920. Only the five crosses in which the number of rows exceed 100 will be considered in detail, as the others are too few in number for analysis. The others may be discussed briefly as follows: All four of the crosses in which Alaska was the original male progenitor produced a high degree of sterility, which, combined with lack of winter hardiness, made it difficult to obtain large numbers. The figures as given, however, show that there is segregation, as both resistant and susceptible sorts were obtained in every case. Moreover,

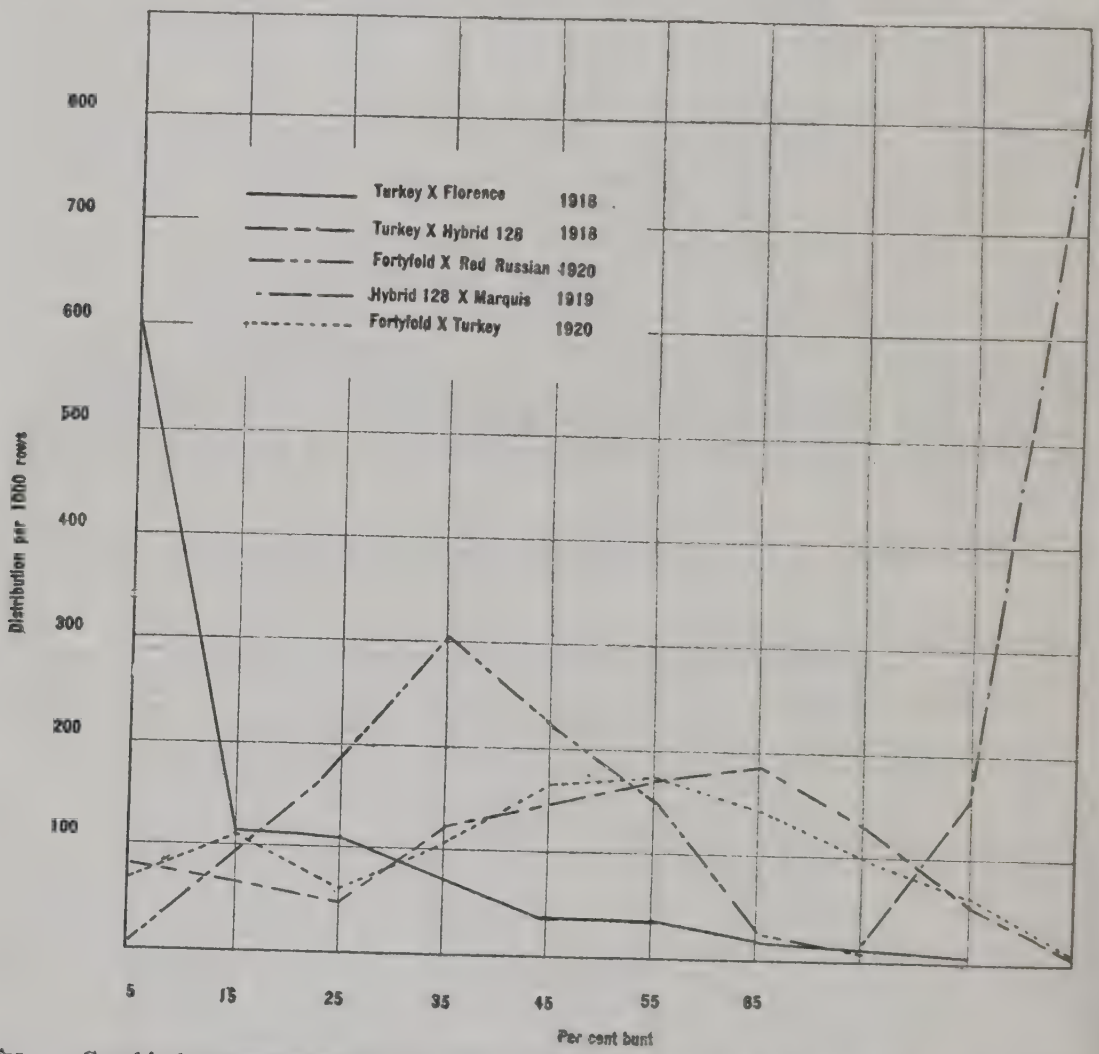

FIG. 1.—Graphical representation of bunt resistance in the F_3 generation of 5 different types of crosses. Three classes of parents were used: Resistant, Turkey (3.5), Florence (7.1); Intermediate, Fortyfold (63.7), Red Russian (61.1); Susceptible, Marquis (74). Hybrid 128 (79.6). Figures in parentheses refer to average percentage of bunt produced under conditions favoring maximum infection.) The crosses of Hybrid 128 with Turkey and Marquis reproduce only the extremes of the parents. In the other crosses transgressive inheritance occurred—that is, segregates were obtained more resistant and more susceptible than the extremes of the parents.

the susceptible classes occur with greater frequency than the resistant ones in the crosses of Alaska and the three susceptible varieties, Jones Winter Fife, Fortyfold, and Red Russian. Crossed with the resistant Turkey, a majority of the F_3 rows are resistant. In fact one of the selections which was sent to the Oregon Station has been found more resistant than either parent there and has proved so promising from a commercial standpoint that it has been continued to the seventh generation. Five F_4 selections and 18 selections in the F_5 generation were grown at Pullman from one F_3 row that was entirely bunt-free, and never a trace of bunt could be found in any later generation, although Turkey

and Alaska, the parent strains, produced a small amount of bunt each year.

An exhaustive test of Turkey × Red Russian was attempted in 1917, but the season was so unfavorable that the grain winter-killed badly and much of it did not germinate till spring, during which time the bunt spores with which the seeds had been inoculated probably perished, for out of 513 rows planted, but 2 produced as much as 10 per cent of bunted heads. Random selection of 42 plants from this F_3 generation gave a preponderance of resistant type in the F_4 generation but was not carried further.

There are five families in which the numbers are sufficiently large and the seasons sufficiently favorable to give results that are dependable for purposes of comparison. The distribution of the different classes has been refigured on the basis of the number of each class per 1,000 rows and the result put in the form of curves in figure 1 in which the ordinates represent the number in each class and the abscissas represent the different classes. There are four distinct types of curves, one in which the 5 per cent class predominates, one in which the 95 per cent class predominates, one in which the 35 per cent class predominates, and two in which the major predominating class is 55 or 65 per cent and a minor class predominating on the resistant end of the curve, and a low center at the 25 per cent class. These two similar crosses are not so similar in reality as they appear from the position of the curves, for the data from which the curve representing the F_3 generation of Turkey × Hybrid 128 was taken was obtained in 1918, while the other curve representing the data of the F_3 generation of Fortyfold × Turkey was obtained in 1920. Reference to Table III shows that the three parent wheats produced an average of 13 per cent more bunt in 1920 than in 1918. If the curves were corrected for this seasonal difference, the F_3 generation of Turkey × Fortyfold would show a parallel curve that would be from 10 to 20 per cent more resistant than the F_3 generation of Hybrid 128 × Turkey. Turkey is one of the parents in both crosses, so the difference in the two curves must be due to the difference in the inheritance of the other two parents, Fortyfold and Hybrid 128. It seems more than coincidence that the 5-year average of the two wheats (Table III) should show 17 per cent less bunt produced by Fortyfold than by Hybrid 128. In fact the F_3 generation of Fortyfold × Turkey shows a mean of 2.3 per cent less bunt than the mean of the other F_3 generation nothwithstanding the more favorable season for bunt in which it was grown. Any combination of the parents in question shows from 10 to 15 per cent more bunt in 1920 than in 1918. The seasonal differences of each parent is, for Turkey, 7 per cent, for Hybrid 128, 14,6 per cent, and for Fortyfold, 15.6 per cent. It seems, therefore, that the curves in question should be separated, on account of seasonal differences, by at least one class, or somewhere between 7 and 15.6 per cent. In other words if the F_3 generation of Turkey × Hybrid 128 had been tested in 1920 instead of 1918 the 194 rows tested would have produced between 7 and 14 per cent more bunt on the average than they did. This would have been between 9.3 and 16.9 per cent more than the F_3 generation of Fortyfold × Turkey actually did produce in 1920. The picture of the inheritance of these two crosses is one in which susceptibility is dominant, with the susceptible segregates fluctuating around the mean of the susceptible parent in each case. A study of the material in the field revealed an important difference

in the two F_3's that the figures in Table IV or the curves in figure 1 do not show clearly, namely, that transgressive segregation occurred in the F_3 generation of Fortyfold × Turkey, there being five rows decidedly more resistant than Turkey and many rows more susceptible than Fortyfold. This phenomenon was not observed in the other F_3 generation, in 1918. Turkey was just as resistant and Hybrid 128 just as susceptible as the extremes of the segregates. This suggests the idea that Fortyfold carries an element of resistance different than the elements of resistance in Turkey, which, added to it, produces a wheat more resistant than Turkey, and without which the segregate becomes as susceptible as Hybrid 128. In susceptible segregates this Fortyfold element of resistance has a value of from 10 to 20 per cent, but in resistant segregates the value lies between 1 and 7 per cent, depending upon the season. It will require tests in later generations to establish this hypothesis beyond question, and this is being done.

It is difficult to place these phenomena of inheritance on a factorial basis on account of the seasonal fluctuations and the quantitative and comparative nature of the material. It is quite evident, nevertheless, that Turkey has several times as much resistance to bunt as Fortyfold. It is also evident, from the performance of the segregates of Turkey × Hybrid 128 in the F_4 and F_5 generations, that this "Turkey resistance" splits up into its component parts when crossed with other wheats of different constitution. Table V shows the comparative resistance of 144 F_4 rows (the offspring of 144 F_3 plants, selected from nine different F_3 rows), in comparison with the parent rows from which they came.

The nine F_3 rows from which the selections were made were the most resistant segregates of beardless, club, winter hardy type (characters which were also being studied). It has been shown in an earlier paper (*14*) that resistance in this cross is not linked with the external characters studied. In comparing the third column, "Bunt in F_3 generation" with the others, it must be borne in mind that these figures were obtained in 1918 and that the F_4 percentages were obtained in 1919, in which year (according to Table III) the parents showed 18.4 per cent more bunt than in the year in which the F_3 generation were tested.

TABLE V.—*Distribution of bunt resistance of Turkey × Hybrid 128 in the F_4 generation from 9 resistant F_3 rows*

F_3 row No.	Number of plants selected from each row.	Average number of F_4 plants per row.	Bunt in F_3 generation.	Average bunt of F_4 rows.	Range of bunt in F_4 rows.	
					Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1387	26	71	1.9	12.7	5.6	35.0
1304	22	56	4.8	18.7	6.5	39.6
1310	2	50	6.4	32.0	25.9	38.1
1353	14	66	6.6	13.6	5.6	25.4
1312	29	63	7.0	13.2	4.3	26.7
1284	12	70	7.1	25.3	12.7	40.3
1297	14	42	9.8	33.2	11.6	59.5
1357	8	69	13.4	26.6	16.6	41.7
1299	17	46	14.6	34.8	15.5	54.7
Average	16	59.2	8.0	23.3	11.6	40.1

The season of 1919 was very favorable, and an average of 60 plants per row was obtained. Four of the 9 F_3 segregates produced F_4 progeny almost as resistant as the resistant parent, Turkey. The range of bunt produced by the different F_4 rows indicates that the F_3 row from which they were selected was probably heterozygous for resistance in every case, the range being from 12.2 to 39.2 per cent, which is a larger fluctuation than Turkey. (Ten rows of Turkey were grown in this same series, for comparison, the extremes of variation being 0.7 and 6.9 per cent.) There was, however, not one of the 144 rows in which the plants did not evidence some of the resistance of the Turkey parent, for the susceptible parent, Hybrid 128, growing alongside of the F_4 rows contained 98 per cent of bunt, whereas the worst F_4 row produced less than 60 per cent. The difference was even more outstanding in the field than the figures indicate. Taxonomically the F_4 rows resembled very closely the susceptible Hybrid 128, but the bunt-infected plants were very different. Once a plant of Hybrid 128 became infected it seldom produced any wheat. Out of 125 infected plants but 3 produced wheat, while 25 of the F_4 rows produced some heads of wheat on every one of the infected plants, and only 11 of the 144 F_4 rows contained more than 10 wholly smutted plants.

The plants in 5 of the 29 F_4 rows from F_3 row No. 1312 were superior in yield and quality and were very resistant; they became the progenitors, therefore, of the F_5 selections, five F_4 plants being selected from each row. Five plants were also selected from each of two other F_4 rows for test in the fifth generation. Thus the individuals of 35 F_5 rows representing 3 F_3, and through them 7 F_4 families were tested for bunt resistance in 1920. Ten of these rows—that is, 2 of the F_4 families—were selected for susceptibility instead of resistance to see whether the amount of bunt could be increased by selection after two generations of selection in the opposite direction. The data are summarized in Table VI.

TABLE VI.—*Distribution of bunt resistance in the F_5 generation of Turkey × Hybrid 128 selected from five resistant and two intermediate F_4 families*

F_4 row No.	From F_3 family row No.	Average number of F_5 plants per row.	Bunt in F_4 row.	Average bunt of 5 F_5 rows.	Range of bunt in F_5 rows.	
					Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1434	1312	42	4.3	13.3	3.3	22.2
1470	1387	42	7.5	19.4	16.1	22.9
1425	1312	52	7.9	9.1	3.1	15.2
1441	1312	59	8.2	18.3	9.2	35.8
1445	1312	48	8.5	14.7	9.1	18.8
1426	1312	60	27.7	23.2	10.8	37.8
1392	1299	45	37.5	48.3	28.8	67.7

The five plants selected from row 1470 were apparently homozygous for resistance in the F_4 generation but lacked an element of resistance that Turkey possesses, for they varied around the mean 19.4 per cent while Turkey in 1920 varied around the mean 7.6 per cent—the worst year for Turkey since the tests began in 1914. The numbers 1426 and 1392 of Table VI show that they were heterozygous and that susceptibility can be increased by selecting in that direction, for one row was within 10 per cent of being as bad as Hybrid 128.

The total populations of the F_4 and F_5 generations are presented in figure 2 in terms of the distribution of rows in the different classes per thousand, similar to the F_3 distribution in figure 1. It will be seen that the F_4 and F_5 generations very nearly parallel each other. If the two susceptible lines had been eliminated, the other five would show decidedly greater resistance than the F_4 generation in spite of the fact that resistant varieties generally were twice as badly smutted in 1920. (See Table III.) Figure 2 may be taken to fairly represent the first peak of resistant segregates of the F_3 curve in Figure 1. No doubt subsequent selections will isolate the full resistant qualities of Turkey in the progeny in the homozygous condition. It is not unlikely that some of the F_5 rows are already in that condition and can not be changed by selection.

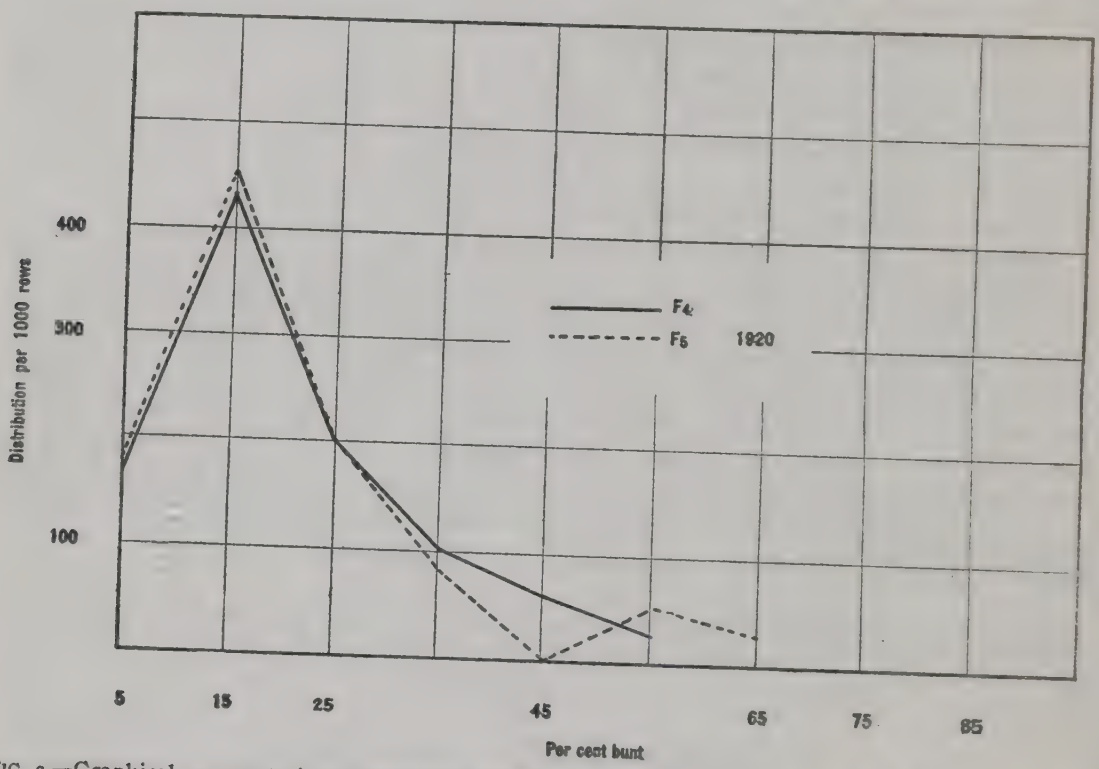

FIG. 2.—Graphical representation of resistant segregates in later generations. The similarity of the F_4 and F_5 segregates indicates the constancy of the selections. The increase on the susceptible end of the F_5 curve is due to the selection of 10 families in that direction from the F_4.

For convenience in summarizing the data in respect to the bunt resistance of Turkey and Hybrid 128 and the progeny of a cross between them, Table VII has been prepared. It should be kept in mind that the result of a given generation represents the constitution of the generation preceding. Thus the results of the F_3 generation show that the F_2 generation reproduced the extremes of the parents with a rather indefinite frequency center near each parent center. The great majority tended toward the center of Hubrid 128, indicating dominance of susceptibility. Similarly the F_4 and F_5 class frequencies indicate the constitutional resistance of the F_3 and F_4 generations. Each later generation shows less variation than the one preceding, as would be expected in a self-fertilized race when selected in the direction of the recessive class center.

TABLE VII.—*Frequency distribution for resistance to bunt in a cross between two varieties of wheat, one resistant, the other susceptible*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Turkey	1918	0.5	7	....	....	....	....	....	....	....	....	....
	1919	.6	11	....	....	....	....	....	....	....	....	....
	1920	2.4	5	....	....	....	....	....	....	....	....	....
Hybrid 128	1918	Not tested.	....	....	....	....	1	5	....	2	....	....
	1919	63.0	....	....	....	....	....	....	....	....	....	2
	1920	98.1	....	....	....	....	....	....	1	3	3	....
T×H F_2	1918	Not tested.	....	....	....	1	....	1	....	1	....	....
T×H F_3	1918	.....do.....	15	13	9	23	28	33	36	25	11	1
T×H F_4	1919	1.9	12	10	3	1	....	....	....	....	....	....
		4.8	1	12	6	3	....	....	....	....	....	....
		6.4	....	....	1	1	....	....	....	....	....	....
		6.6	3	10	1	....	....	....	....	....	....	....
		7.0	8	16	3	....	....	....	....	....	....	....
		7.1	....	5	3	3	1	....	....	....	....	....
		9.8	....	2	5	2	3	2	....	....	....	....
		13.4	....	4	1	2	1	....	....	....	....	....
		14.6	....	2	6	3	4	2	....	....	....	....
T×H F_5	1920	4.3	1	3	1	....	....	....	....	....	....	....
		7.5	....	3	2	....	....	....	....	....	....	....
		7.9	3	2	....	....	....	....	....	....	....	....
		8.2	1	3	1	....	....	....	....	....	....	....
		27.7	....	2	2	1	....	....	....	....	....	....
		37.5	....	....	1	1	....	2	1	....	....	....

Table VIII gives the class frequencies of Turkey and Florence in respect to bunt resistance as well as those of the progeny obtained from a cross between them. An extra column, not given in Table VII, has been added to show the rows that were entirely without visible infection when harvested.

It will be seen that the F_2 generation (the constitution of which is shown by the F_3 class frequencies) shows transgressive inheritance, with the greatest frequency coinciding with the average resistance of the parents, if it be assumed that the 72 immune segregates varied in the intensity of their resistance in proportion to those in the direction of the susceptible end of the table. Such an assumption seems warrantable from the records of the F_4 and F_5 generations, for they were more resistant than the parents, and only a trace of bunt appeared in each generation in a few of the rows. The F_5 generation shows that 19 out of 25 rows were bunt-free (immune), and their ancestry had been bunt-free since the cross was made. Thus, for three generations these selections have not produced a trace of bunt, although planted each year under conditions favoring maximum infection—that is, the seed was blackened with spores. The parents produced an average of 3.5 and 7.1 per cent under the same conditions, and other varieities, classed as susceptible produced as much as 80 per cent of bunt.

TABLE VIII.—*Frequency distribution for resistance to bunt in a cross between two varieties of Triticum vulgare, both of which are resistant*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—									
			Immune.	5	15	25	35	45	55	65	75	85	95
		Per cent.											
Turkey 326	1918	0.5	1	6	....	....	....	....	....	....	....	....	....
	1919	.6	.....	11	1	....	....	....	....	....	....	....	....
	1920	2.4	.....	5	....	....	....	....	....	....	....	....	....
Florence 634	1918	.0	1	2	....	....	....	....	....	....	....	....	....
	1919	.9	.....	2	....	....	....	....	....	....	....	....	....
	1920	8.1	.....	1	....	2	....	....	....	....	....	....	....
326×634 F_2	1918	Not tested.	.....	1	1	....	....	....	....	....	....	....	....
326×634 F_3	1918	.....do.....	72	29	19	18	12	6	6	3	2	1	....
326×634 F_4	1919	.0	11	1	....	....	....	....	....	....	....	....	....
		.0	9	2	....	....	....	....	....	....	....	....	....
		3.2	3	8	....	....	....	....	....	....	....	....	....
		[a] 67.7	3	....	....	....	....	....	....	....	....	....	....
		.0	2	5	....	....	....	....	....	....	....	....	....
326×634 F_5	1920	.0	3	2	....	....	....	....	....	....	....	....	....
		.0	5	....	....	....	....	....	....	....	....	....	....
		.0	4	1	....	....	....	....	....	....	....	....	....
		.0	5	....	....	....	....	....	....	....	....	....	....
		.0	2	3	....	....	....	....	....	....	....	....	....

[a] This is evidently a mistake. The row next to it was immune, and it seems probable that the numbers got mixed at harvest time, for the three selections showed no trace of bunt the following year.

In 1919 an F_3 family of Hybrid 128 × Marquis was produced to test the inheritance of the resistance of Marquis to bunt. Information regarding the inheritance of Hybrid 128 had been obtained the year before from the F_3 generation of the cross between this variety and Turkey. The question arose as to whether the seed should be planted in the fall or spring, one parent being a winter wheat the other a true spring variety. If fall-sown, much winterkilling would be expected, for Marquis is not very winter-hardy. If spring-sown the true winter segregates would not produce heads and two-thirds of the others would be heterozygous for the winter factor and would produce some winter plants and some that would develop late so as to interfere with harvesting operations. The former course was decided upon and the planting was done in October, 1918. The winter was unusually mild, and a good stand was harvested the following summer. Every one was surprised to find all of the F_3 rows (334 in number) very badly bunted. The best row in the lot was three-fourths smutted, and 276 of the rows produced more than 90 per cent of bunt. The whole block of rows appeared as a mass of stinking smut which gave off an offensive odor that penetrated to windward for half a mile. An examination of some demonstration plots in another field showed that the fall-sown Marquis was very smutty. This gave a clue as to why all of the F_3 rows of Hybrid 128 × Marquis were badly bunted. The inherent resistance of Marquis was broken down by the winter rest period.

One plant each from nine of the best rows—that is, those having the least amount of bunt—and one from each of the four worst rows were selected for testing in the F_4 generation. The complete results of the parents, the F_2, F_3, and these F_4 selections are summarized in Table IX.

TABLE IX.—*Frequency distribution of resistance to bunt in a cross between Hybrid 128 and Marquis, both of which are susceptible when fall-sown*

Variety.	Year tested.	Bunt in parent row.	Number falling into class with average percentage of bunt of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Hybrid 128	1919	63.0										2
	1920	98.1							1	3	3	
Marquis	1919	Not tested.								1		
	1920	74.0				1	[a] 3		[a] 1			
H × M F_2	1920	Not tested.						1				
H × M F_3	1919	do								5	53	276
H × M F_4	1920	75.9						1				
		76.7									1	
		78.6					1					
		79.4						1				
		80.9								1		
		81.1						1				
		81.3					1					
		83.0								1		
		83.9								1		
		94.2						1				
		96.9								1		
		97.6							1			
		98.4									1	

[a] Seed from a different source, but since Marquis is a variety recently introduced and appears to be constant for all its characters, it is likely that there is no genetic difference in the resistance of these strains, for seed from these same strains planted in the spring produced less than 5 per cent of bunt.

The F_4 generation shows an average of 19.9 per cent less bunt than the F_3 parent rows, but this is undoubtedly due to seasonal fluctuations, for Hybrid 128 and Marquis showed similar differences in 1919 and 1920. A closer study of the F_4's indicates that even in these extremely susceptible segregates there is a constant difference in their susceptibility comparable to the differences in the susceptibility of the parents. This is more clearly brought out by the following figures:

	Average percentage of bunt.		Difference.
	1919	1920	
From 9 low selections	80.2	62.2	18.0
From 4 high selections	96.8	72.4	24.4
Difference between high and low	16.6	10.2	6.4

Out of the 9 low F_3 rows (between 75 and 84 per cent bunt) 5 produced the smallest percentage of the 13 rows in the F_4 generation. Out of the 4 high F_3 rows (between 94 and 99 per cent bunt) selected, one produced the highest amount of bunt in the F_4 generation, and two of the others produced more than the average of all the F_4's in 1920. This indicates that Marquis carries a weak factor or property for resistance that is inherited and is not affected by fall planting. If this be true, strains from this cross could be selected that would show a consistent difference

of from 15 to 30 per cent when planted in the fall under conditions favoring maximum infection. In 1919 Hybrid 128 produced 98 per cent, Marquis produced 75 per cent, and the F_3 varied from 75 to 100 per cent. In 1920 the parent varieties produced 30.9 and 74.3 per cent respectively, an F_2 progeny produced 56 per cent of bunt, and the F_4 selections varied from 46 to 85 per cent. Five plants, each selected from the lowest and highest F_4 row, produced an average of 78 and 91 per cent bunt in the F_5 generation in 1921. These figures include only the rows that were planted together in the same part of the field, and on the same date. There is much work to be done to establish the inheritance of the resistance of Marquis, especially the part played by the winter rest period, the segregation of the units that are not so affected, and the complex of its resistance for spring planting. This is being done and will be reported later.

There remains one other cross to discuss, that of Fortyfold × Red Russian. The average susceptibility of the parent varieties and their F_3 offspring in 1920 was 36.7, 49.3, and 37.5 per cent, respectively. The F_3 segregates varied from 8.1 to 73.6 per cent. Reference to figure 1 shows the greatest class frequency of the 181 F_3 rows to be between 30 and 40 per cent, with rapidly diminishing class frequencies above and below. The most striking thing about this cross is that the average of the F_3 generation is less than the average of the parents. The fact that the parents were duplicated so that there were nearly 200 plants counted precludes the possibility of a chance error. In the other four crosses charted in figure 1 the average of the parents lie below the average susceptibility of their respective F_3's, showing dominance of susceptibility. In this cross there seems to be a slight dominance of resistance, the parents producing on the average 5.6 per cent more bunt than the F_3 progeny.

The lower limit is decidedly lower than any recorded example of either parent under similar conditions. This shows that Fortyfold and Red Russian (which are known to be intermediate in respect to resistance of bunt, when compared with wheats like Turkey, Alaska, and Florence on the resistant side, and Hybrid 128 and Jones Winter Fife on the susceptible side) possess different kinds of resistance which are cumulative in effect, when brought together by crossing. These resistances have a value somewhere between 10 and 20 per cent in reducing the amount of bunt that would be produced on the extremely susceptible varieties when planted under conditions favoring maximum infection. By crossing them, segregates are obtained that are from 10 to 20 per cent more resistant than either parent. It is to be expected, and the evidence points to the probability, that segregates also occur which are from 10 to 20 per cent more susceptible than either parent. The difficulty of isolating the susceptible segregates, on account of the fungus destroying the reproductive organs, was pointed out in another place. The segregable dilute or feeble resistance of Fortyfold shown here is in accord with that found in the cross between it and Turkey. The feeble resistance of Red Russian has not as yet been definitely corroborated in other crosses.

DISCUSSION AND CONCLUSIONS

The investigation described in this paper forms a part of the general work of the Washington Experiment Station looking toward the discovery of methods for reducing the destructiveness of bunt or stinking-smut in

wheat. The particular phase treated is the development of a commercially desirable wheat which will not develop bunted heads even on a badly infected soil. Emphasis has been placed on inheritance of bunt resistance, but the ultimate practical application of the principles involved has been continually kept in mind. (Pl. 3, B.)

The first tests for resistance were made in 1914 when a few of the common commercial varieties were tested for comparative resistance under conditions favoring maximum infection. During the next two years, while these and other varieties were being tested in greater detail, methods of infecting the seed, recording the data, and computing the resistance on a comparative quantitative basis were developed and standardized. In an endeavor to obtain the best possible material for the breeding work, more than 500 named varieties, from the principal wheat districts of the world, including representatives from the eight species, have been tested for resistance.

Most of the varieties tested proved to be either very susceptible or lacking in winter hardiness. Thus the representatives of the four species, einkorn, polish, spelt, and emmer, although all very resistant, were discarded because they were of no commercial value, either from the standpoint of yield or hardiness. One poulard (Alaska) was used as the male parent in several crosses, mainly because of its bunt-resisting qualities. One club (Hybrid 128) was selected for two of the crosses described below because of its stiff straw, early maturity, and prolificacy, notwithstanding its malignant susceptibility to bunt. The other six varieties were selected for the breeding work from the vulgare group because they were common commercial varieties (with the exception of Florence, which was selected for its exceptional resistance) in the Northwest, and contained the greatest range of desirable characteristics.

Alaska, Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones' Winter Fife have the winter habit of growth; that is, they will not head out if planted in the spring, and are considered sufficiently winter hardy for fall sowing. This is of prime importance because the chief losses due to bunt occur in winter wheat. Marquis and Florence are spring wheats and are not considered sufficiently hardy for fall sowing, but were such good milling wheats and so resistant to bunt that they were used for the additional purpose of studying the inheritance of winter hardiness.

These eight varieties (Pl. 2, B; 3, A) have been grown as pure lines from individual plant selections for 6 to 15 years and from the first have bred true for their morphological characters and may be assumed to be constant, within the limits of soil and seasonal fluctuations, in their comparative resistance to bunt.

Turkey is one of the most resistant of all the wheats tested, seldom producing above 5 per cent of bunted heads, even under the most favorable conditions for infection. From one-fourth to one-half of the plants may show infection, but only a few flowers, or at most only part of the heads on an infected plant produce smut balls, the remainder producing normal wheat. The other resistant varieties, Alaska, Florence, and Marquis, show similar characteristics in that only a small part of the flowers on the infected plants are destroyed by the fungus. The two very susceptible varieties, Hybird 128 and Winter Fife, on the other hand, do not normally produce anything but smut balls on the infected plants, the number of flowers or heads escaping being negligible compared with those that are destroyed. An occasional plant escapes infection altogether and produces normal grain; but once the parasite establishes itself within the

host, more than 80 per cent of the plants on the average produce only the fruiting bodies of the parasite, and the others produce less than 40 per cent of grain. Fortyfold and Red Russian, which are intermediate in respect to total bunt produced, are also intermediate in respect to the amount of wheat produced on the infected heads.

The resistance of Turkey is different from that of Alaska, for segregates of a cross betwen them occurred which were more resistant than either parent, inasmuch as no trace of bunt could be obtained from the F_3 to the F_7 generation, although planted under conditions favoring maximum infection—conditions under which both parents showed traces of bunt on more than one-fourth of the plants. Such a result would be impossible unless the resistance of the two wheats were cumulative in effect, each contributing something that the other lacked. For if the resistance were the same in both parents, the offspring would fluctuate around the same mean and no segregates would occur more resistant or more susceptible than the parents. If, however, the resistance of Turkey and Alaska were due to different causes, or perhaps the genes located in different chromosomes, then the offspring would show greater variation than the parents, and segregates would occur which would be permanently more susceptible than either as well as more resistant. If the resistance of each parent were composed of differing multiple factors, many new and different segregates would occur. Thus, if Turkey resistance *abc* met Alaska resistance *xyz*, and each factor reduced the amount of bunt by 20 per cent, a cross between them should give varients (assuming the susceptibility of the parents separately to be 5 per cent) in the F_2 generation that would produce bunt as follows:

Number of resistant factors.	Bunt produced.	Classification.	Number of resistant factors.	Blunt produced.	Classification.
	Per cent.			*Per cent.*	
0.....	65	Nonresistant.	3.....	5	Very resistant.
1.....	45	Slightly resistant.	4.....	−15 o	Immune.
2.....	25	Somewhat resistant.	5.....	−35 o	Do.
			6.....	−55 o	Do.

Unfortunately the large number of sterile plants and flowers produced by crossing Turkey and Alaska, combined with the lack of winter hardiness, reduced the number of plants to such a degree that an accurate analysis of the particular type of segregation that occurred was impossible.

Florence is a very resistant spring wheat, but its resistance is also different from that of Turkey, for transgressive inheritance occurred in a cross between them, F_3 segregates occurring all the way from completely immune to completely susceptible individuals, not unlike the illustration given above. More than 40 per cent were immune, while the plants in 12 rows (7 per cent of the F_3 progeny) were more than 50 per cent bunt, an amount never produced by either parent under the most favorable conditions for infection. Something like 30 per cent of the rows were intermediate, producing from 10 to 50 per cent of bunted heads.

It is not too much to suppose that the 72 immune rows differed in the intensity of their resistance, but there is no way of proving it except by the slow, laborious process of crossing them with susceptible varieties

and testing the comparative resistance of their descendants. The intermediate and susceptible segregates indicate multiple factors for resistance in both Turkey and Florence, the loss of which renders a given hybrid offspring in a later generation a congenial host for the parasite. Such factors added together are cumulative in effect, making the segregate possessing them immune against all attempts of the organism to set up a pathogenic relationship.

Fortyfold has a dilute or weak resistance that is evidently different from that of any of the resistant elements of Turkey, for in the F_3 generation of Fortyfold × Turkey five out of 593 F_3 families occurred that were distinctly more resistant than Turkey, and many occurred more susceptible than Fortyfold. This dilute resistance of Fortyfold showed a value of approximately 5 per cent in 1920 when added to the resistance of Turkey in the very resistant F_3 families, but in the absence of it and also the Turkey resistance, the segregates increased 15 to 20 per cent in the amount of bunt produced. High and low selections are being tested in the F_4 generation in 1921 to determine the correctness of these assumptions, for the data from the F_3 progeny are not as conclusive as one would like to have in a characteristic which shows such wide fluctuations as bunt resistance.

Hybrid 128 has no heritable resistance distinct from that of Turkey or Marquis, for the F_3 progeny of crosses with them did not give variations exceeding the extremes of the parent types. The intermediates in the F_3 generation of Turkey × Hybrid 128 indicated that the resistance of Turkey was made up of multiple factors, which, when separated, gave dilute resistances. The 334 F_3 families of Hybrid 128 × Marquis were all very smutty, none having less than 70 per cent of bunt. In the F_4 generation, however, the high selections gave 10 per cent more bunt than the low ones, which indicates a dilute resistance, probably of Marquis, that is not affected by the winter weather. Marquis is very resistant when spring sown, but this resistance is mostly destroyed or neutralized by the winter rest period when fall sown.

The resistance of Red Russian is not well established in the cross with Turkey on account of the small numbers and the unfavorable season in which it was tested, but in so far as the F_4 selections tell anything they indicate that any resistance it may possess is not different from that of Turkey, for none of the segregates were more resistant than Turkey, and the most susceptible did not surpass Red Russian in amount of bunt produced. The cross between Fortyfold and Red Russian, however, prove that the latter has a definite heritable resistance which, added to the unlike resistance of Fortyfold, produces segregates much more resistant than either parent. That is, the two weak resistances when brought together have the effect of a strong resistance comparable to that of Turkey or Alaska. The feeble or weak resistance of Fortyfold is in accord with that found in the cross between it and Turkey. The dilute or feeble resistance of Red Russian has not as yet been corroborated in other crosses in sufficient detail to warrant discussion.

From the foregoing evidence, based on seven years' work with bunt resistance in wheat, and with special reference to eight varieties, and the inheritance of resistance in the progeny of crosses between them, the general conclusions may be summarized as follows:

The most susceptible wheats, planted under conditions favoring maximum infection, produce an average of about 80 per cent of bunted

heads. Hybrid 128 and Jones Winter Fife belong to this class. Although they produce 20 per cent of sound heads, this seems to be due to accident, for in crosses with other varieties the descendants do not show evidence of having inherited any cumulative resistance whatever from these varieties.

Fortyfold, Red Russian, and Marquis each have differing dilute resistances which reduce the amount of bunt by 10 to 25 per cent. When added together, as in descendants of crosses between them, the cumulative effect makes a more concentrated resistance, having a value of from 30 to 60 per cent. In addition to the dilute resistance, Marquis has a strong winter-sensitive resistance with a value of 50 to 60 per cent in spring-sown grain which is ineffective in preventing bunt when the seed is fall sown. That is, if Marquis is sown in the fall, only the dilute resistance is operative, for it produces but 10 to 20 per cent less bunt than the most susceptible varieties; if sown in the spring, the strong winter-sensitive factor becomes functional, and the resulting crop produces 60 to 75 per cent less bunt than the completely susceptible varieties. There is some evidence that all the different resistances are somewhat winter-sensitive, for the facultative wheats (those that may be sown either in the fall or in the spring, being true spring wheats but fairly winter hardy) are known to produce more bunt from fall seedings. Florence also registers a higher degree of bunted heads in the fall-sown tests.

Turkey, Florence, and Alaska each have differing concentrated resistances which reduce the amount of bunt 70 to 75 per cent, compared with the standard susceptible varieties. These concentrated resistances are also cumulative in effect when brought together by crossing, the resulting descendants segregating into immune, very resistant, various stages of dilute resistant, and completely susceptible classes.

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PLATE 1

A.—Result of a smut explosion. Machine of W. J. Grier, near Colfax, Wash., a few hours after the explosion, August 12, 1915. By hard fighting the sacked grain on the right was saved, but the machine and straw stack were burned. Hundreds of thrashing outfits and thousands of bushels of wheat have been destroyed in the Pacific Northwest due to fires following explosions of smut dust (bunt spores), which burn like powder.

B.—Morphological effect of bunt on wheat heads. The three on the left are Alaska, the others Fortyfold. Heads of each variety from left to right contain normal seed, both normal seed and smut balls, and only smut balls. In Alaska the parasite causes the awns to fall but dwarfs the head instead of stimulating increased length as in Turkey and Hybrid 128. (See Pl. 2, A.) The head length of Fortyfold is not affected, but the spikelets on the infected heads stand out more nearly at right angles to the rachis.

A

B

A

B

PLATE 2

A.—Morphological effect of bunt on wheat heads. The three on the left are Turkey, the others Hybrid 128. First head on the left, normal grain; second head, half wheat and half bunt; third, all bunt. The bunted heads are much elongated as if trying to grow away from the fungus. In Turkey the awns fall off prematurely on the bunted heads. The bunted head of Hybrid 128 on the extreme right looks like a vulgare instead of a compactum.

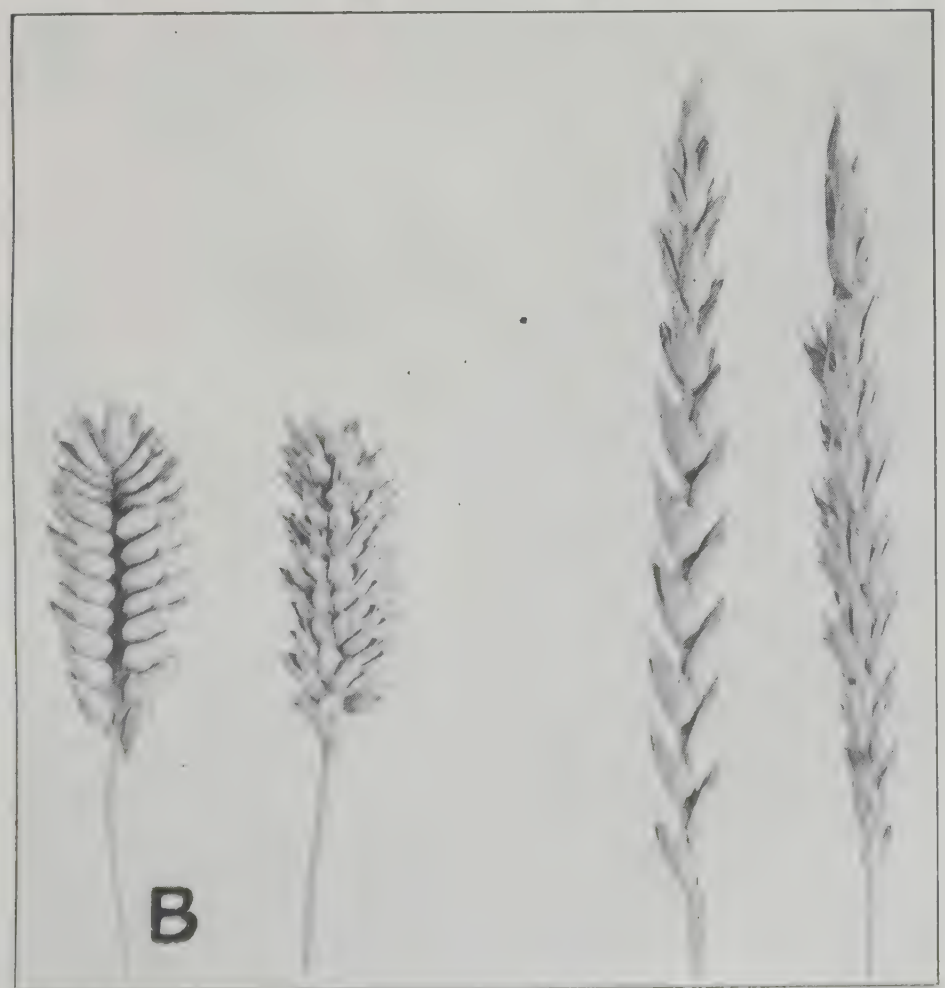
B.—Parent varieties resistant to bunt. From left to right, Turkey, Florence, Marquis, Alaska. Turkey and Alaska are true winter wheats and can not be used for spring sowing. Florence and Marquis are typical spring wheats. Marquis and Turkey are the most popular varieties in the United States and Canada, measured by the quantity produced. Both are excellent breadmaking wheats.

PLATE 3

A.—Parent varieties susceptible to bunt. From left to right, Fortyfold, Jones Winter Fife, Red Russian, Hybrid 128. Jones Winter Fife and Hybrid 128 are about 20 per cent more susceptible than the other two. All are true winter wheats of commercial importance in the Northwest. Fortyfold and Hybrid 128 have white grain.

B.—Two hybrid (F_5) types with commercial possibilities. Left, the morphological characters of the Hybrid 128 parent are combined with the resistance of Turkey. On the right the Florence head type is combined with the winter hardiness of Turkey. It has been immune to bunt for three years. Both selections outyielded the parents in 1919 and 1920.

A


B

A BACTERIAL LEAFSPOT OF TOBACCO[1]

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INTRODUCTION

During the past five years a bacterial leafspot of tobacco occuring in Wisconsin has been the subject of investigation together with observations on tobacco leafspots in general in various tobacco districts of the United States. The tobacco leaf is subject to an exceptionally large number of diseases, originating from a variety of causes, in some cases with distinctive and constant symptoms, but more often confusing when any determination or classification based on symptoms is attempted. This confusion is in some respects magnified by the fragmentary early literature and is due largely to common names having been applied without adequate consideration as to the causal agents of the diseases. Recently two or three leaf diseases have been described, however, in sufficient detail to permit of definite reference. It is the purpose of this paper to contribute to the knowledge of one other such disease.

CAUSE

This disease, which is not believed to be new to tobacco growers in Wisconsin, has been found to be due to a bacterial organism apparently previously undescribed. The causal organism has been named *Bacterium melleum*, n. sp., and a description of the organism and the symptoms of the disease it causes is given in this paper.

COMMON NAMES

The leafspot to be described here is ordinarily called "rust" by Wisconsin tobacco growers. This name, which is not a good one from a phytopathological standpoint, is in fact in use throughout most of the tobacco districts of the world, for a variety of leaf diseases of tobacco, and is, therefore, a very unreliable term. The term "rust" is frequently limited by the use of such combinations as "red," "brown," "white," and "black rust;" but these again are of little significance when applied by different individuals and have little definite relation to the causal agent concerned. Other terms such as "firing," "black fire," field fire," "wildfire," "speck," and "frogeye" are terms which have been commonly used as synonymous with "rust," though the term "frogeye" is now generally limited to the disease caused by *Cercospora nicotianae*

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El. and Ev. and more recently "wildfire" has become the accepted common name for the disease caused by *Bacterium tabacum* Wolf and Foster.

The leafspot diseases of tobacco naturally fall into three disease categories, as regards cause, namely, (1) those due to nonparasitic agents, (2) those due to fungi, and (3) those due to bacteria. It is probable that additional terms should not be added to the list of common names until a satisfactory basis of classification based on these categories is established. However, we have come to refer in the laboratory to the disease herein described as the "Wisconsin bacterial leafspot" disease, and this term may tentatively be preferable to the commonly used term "rust."

OTHER BACTERIAL LEAFSPOTS

Although it is only within recent years that certain leafspots of tobacco have been definitely shown to be of bacterial origin, it is fairly certain that one or more have existed from the earliest days of tobacco culture in this country. The earliest treatises on tobacco culture refer to "rust" and "firing," although in most cases it would be difficult to judge the nature of the causative agent from the descriptions of these diseases. Killebrew and Myrick (*6*),[2] for instance, wrote as follows—

> another field fire, called "black fire," which is totally different from the red field fire, is caused by excessive humidity and occurs only after continued rains of several days' duration with hot weather. This black fire is much more to be dreaded than the brown rust or red field fire, for it attacks the plants while *immature*, involving all the leaves. Sometimes the disease will spread over a field in two or three days and ruin the crop, making black deadened spots as large as a silver dollar, but this rarely happens.

This disease was undoubtedly parasitic in nature, especially in view of the fact that these experienced observers separated it from other symptoms probably of nonparasitic origin. It is also quite likely that this disease was one of the two tobacco leafspot diseases recently shown to be of bacterial origin in this country.

Similarly the disease described in this paper as "Wisconsin leafspot" has probably existed in Wisconsin for 50 years or more along with other leafspots under the name of "rust," but now referred to by some of the older growers as "old-fashioned rust," on account of the fact that it has not been as prevalent in recent years as in the earlier days of the industry in this State.

Apparently the first leafspot disease of tobacco attributed to bacteria was that of "la rouille blanche" (white rust) of France, ascribed by Delacroix (*1*) in 1905 to *Bacillus maculicola*. The description of this disease is not sufficient to afford adequate comparison with our American leafspots, but in any case the description given indicates that it is different from the Wisconsin bacterial leafspot. Honing (*4*) in 1914 described a rust occurring in Deli (Sumatra) which he showed to be due to a bacterial organism which he named *Bacterium pseudozoogloeae*. This disease was also known as "black rust," although it evidently was not the "wildfire" disease of America, since neither the description of the causal organism nor that of the symptoms of the disease correspond with that of wildfire. Honing's disease corresponds more closely to that of the Wisconsin leafspot, although, as will be shown by later comparison, they differ in several respects.

[2] Reference is made by number (italic) to "Literature cited," p. 492–493.

Wolf and Foster (7) in 1917 described the wildfire disease as it occurred in North Carolina and Virginia, following an unusually severe outbreak and proved the causal organism to be a bacterium, which they named *Bacterium tabacum*. This disease apparently has since spread to most of the other tobacco districts in the United States, seemingly from the North Carolina epidemic of 1917 as a center of infection. This disease is not readily distinguished from the Wisconsin leafspot in general symptoms, although the chlorotic area around the point of infection is usually larger and more common than in the Wisconsin leafspot. The wildfire organism is also a much more vigorous parasite than the Wisconsin leafspot organism, and the disease may consequently be much more prevalent and serious where it occurs. Fromme and Murray (3) investigated a leafspot disease in Virginia, which had apparently also existed for a considerable time in that State, and found it to be due to a bacterium which they named *Bacterium angulatum*. This disease has been named by them "angular leafspot" on account of the angular shape of the lesions. It cannot be readily confused with other bacterial leafspots but may be difficult to distinguish at times from certain nonparasitic spots when judged by symptoms alone.

DESCRIPTION OF THE DISEASE

The Wisconsin bacterial leafspot has been found ordinarily on the lower leaves of the plants in the field. Usually it is most marked on the lowest leaves but has been observed during this investigation up to the middle leaves. In severe outbreaks, not seen since the beginning of this study but earlier noted and referred to by others as "old-fashioned rust," the leaves on the entire plant may be involved. That the disease may occur on young leaves is evidenced, however, by the observation of several infections in seed beds from which the causal organism has been isolated. The older leaves are seemingly more predisposed to a rapid collapse and death, and finally, browning of the tissue when once infected, but when artificially inoculated by needle punctures the top leaves show equal predisposition to infection, and the development of the chlorotic area surrounding the point of infection is even more marked than on lower leaves. The common occurrence of the disease on the lower leaves in the field is due quite likely to the more favorable environmental conditions offered there for infection and progress of the disease.

The disease in the seed beds ordinarily is inconspicuous and not as typical as in the field. The spots are usually small and more angular than in the field and the chlorotic area less distinct (Pl. 2, A). The old lesions are usually small and light-colored, but when they run together the young leaves present the appearance of being blighted.

In the field the young spots are usually round, frequently with a small central fleck surrounded by a distinct chlorotic area or halo, identical with that of the wildfire disease. Under other conditions this halo may not appear at all or may disappear rapidly, the tissue surrounding the point of infection collapsing and soon turning brown in color, in some cases possibly white. Enlarging spots may or may not be limited by the veins of the leaf. At times the infected area seems to pass over the vein without injury to it. At other times, however, the parasite may enter the vein and follow it, producing an elongated lesion. The diameter of the spots may vary from 1 mm. to 1 cm. or more, frequently coalescing, and hence involving large areas of the leaf. The old lesions

are usually distinctly brown in color, sometimes brownish white, frequently with a dark center giving a "birds-eye" appearance. Concentric rings, usually are not present, though apparently they may occur (Pl. 1, A, B).

An interesting symptom frequently evident in the greenhouse after artificial infection is the formation of a secondary ring of small lesions 2 or 3 mm. beyond the circumference of the primary lesion. This ring, often perfect in shape, seems to follow as a result of renewed activity by the parasite about the primary lesion following a checking of the disease. The chlorotic area surrounding the center of infection has been found to be relatively free of organisms, as was found by Miss Elliott (*2*) in the halo-blight of oats.

PREVALENCE OF THE DISEASE

The causal organism of the Wisconsin leafspot disease was first isolated in the spring of 1917. On account of the similarity of other leafspots, nothing conclusive can be said as to the prevalence of the disease prior to that time, although the writer feels confident that within 20 years of casual observation previous to 1917 he has seen a number of more serious cases of the disease than have been noted since. This belief is strengthened by the testimony of a number of the older tobacco growers in the State, who recall complete losses of portions of crops from "rust," which, from our subsequent observations on nonparasitic leafspots, are not believed to develop to such an extent on the type of tobacco grown in this State, with the possible exception of the "rust" following mosaic. "Rust" following as a result of mosaic is not, however, ordinarily limited to such an extent by the topography of the land and the opportunity for infection as is the bacterial leafspot. Since 1917 a number of mild occurrences of the disease have been seen within a 25-mile radius of Madison, and in most cases have been identified by isolation of the causal organism and inoculation experiments. Search has been made for this disease in a number of other tobacco districts, mostly in Kentucky, Maryland, Pennsylvania, and in the Connecticut Valley. Only one specimen has been collected which can with certainty be said to be the same disease, and this was from Kentucky in 1919. One collection from Connecticut in July, 1919, proved to be the "wildfire" disease, and was the first record of that disease in the Connecticut Valley. Similarly, collections made in 1920 from Maryland, Kentucky, and Ohio proved to be wildfire.

ISOLATIONS

The first isolation of the Wisconsin leafspot organism was made in June, 1917, from the seed beds at the experiment station at Madison. At about the same time specimens of a leafspot on tobacco seedlings (wildfire) were received from Mr. E. G. Moss, in charge of the branch tobacco station, Oxford, N. C. This disease was at first thought to be due to a fungus, and preliminary isolation and infection experiments were conducted from this standpoint with negative results. Bacteria were soon after isolated and infection secured. Word was then received that Dr. Frederick A. Wolf, of the North Carolina Station who was working on the same disease, had established the bacterial relationship, no doubt a few days before our own conclusion had been reached. A few weeks later the writer visited the North Carolina section and had the opportunity of noting a second serious outbreak of the disease in that section.

On his return to Wisconsin similar leafspots were noted, among them certain spots on a row of a southern type of tobacco in the experimental plots at Madison, though not occurring nearly as seriously as in the fields seen in North Carolina. The records show that two of the isolations from this row gave white organisms, one of which was infectious. Unfortunately this culture died before a detailed study could be made of it, so that we are not at all certain that it was the wildfire organism. The writer's earlier isolation from the seed bed leafspots and later isolations from the field yielded, however, only yellow infectious organisms. No white organism has since been isolated except following known cases of inoculation with the wildfire organism. It is felt that this explanation should be made here in view of a statement made by Wolf and Foster (7) as a result of correspondence, to the effect that wildfire occurred in Wisconsin in 1917. While the "similar spot" referred to has developed to be what we now call the "Wisconsin leafspot," there is still some probability that we did have one case of wildfire on a row of southern tobacco in 1917, and if so, it seems likely that it was the result of seed-borne infection. In any case wildfire can not be said to have occurred in Wisconsin in 1917 in the sense that it has since been reported from other States, nor was it introduced in that manner until 1922.

During the last five years a large number of isolation tests have been made from various sorts of leafspots of tobacco. Wherever fairly fresh and young spots of the wildfire disease or the Wisconsin leafspot have been plated out, no difficulty has been encountered in getting pure cultures at once, so that the distinction between these two diseases has been readily established. The method employed has been simply to select a young lesion, cut it out with a scalpel, and rinse it through 8 to 10 sterile water blanks with vigorous shaking. It was then transferred to a tube of bouillon, mashed with a sterile scalpel or rod on the side of the tube, rinsed into the bouillon and allowed to stand 15 to 30 minutes. One to six loopfuls of the bouillon werethen transferred to melted potato agar at about 45° C. and plates poured. Cultures were usually kept in stock on potato-dextrose agar in the ice box. Twelve different isolations of the Wisconsin leafspot organisms have been made over a period of five years. Practically all the cultural character studies were made with one organism (culture No. 141), and where this culture was not used we have made sure that we have used a pathogenic organism corresponding in ordinary cultural characteristics.

INOCULATION EXPERIMENT

A very considerable number of inoculation experiments have been made in connection with demonstrating the pathogenicity of the organism isolated, testing the strains following growth in culture for different periods, and for comparison with other bacterial leafspots of tobacco, particularly wildfire. Repeated trials have also been made comparing inoculation by spraying and by needle puncture, under very variable environmental conditions. It has been found that, while good infection is always secured on wounded leaves with a virulent strain of the Wisconsin leafspot organism, practically no infection at all has ever been secured by simply spraying the plants with a suspension of the organism in water.

The writer can not state with certainty the relation of normal field infection to wounded tissue. He has not been able to find from observa-

tion that wounding by insects or other means has played any part in infection. It seemed rather that infection in the field was dependent upon the occurrence of favorable environmental conditions. Every attempt to duplicate such conditions experimentally has thus far given negative results. The writer has as yet, however, done nothing as regards the intrinsic predisposition of the plant itself to infection, and it is not improbable that the host grown under different conditions as regards chemical and physical relationships may be considerably altered thereby as regards predispostion. This belief is strengthened by the fact that similar experience has been had with frequent attempts at securing good infection with the wildfire organism by spraying under greenhouse conditions, while with this organism we know that under other conditions good infection may be secured in this manner.

The method of inoculation by wounding has been essentially that of puncturing the leaf with a needle point which has been dipped in a suspension of the organism in water and permitting a small droplet of the watery suspension to cover the wound. In this manner, when a virulent strain of Wisconsin leafspot organism is used, infection is secured in two to five days, and symptoms develop which compare favorably with those from *Bacterium tabacum* in size of chlorotic area or lesions obtained (Pl. 2, B). On the other hand, it is certain that under field conditions the Wisconsin leafspot organism is not as virulent as the wildfire organism, and that the former can be pathogenic only under more limited conditions than is the latter. Under field conditions the chlorotic area or halo formed by the Wisconsin leafspot organism is not normally as marked as that of wildfire (Pl. 1, A) and frequently may not occur at all (Pl. 3, A) on older leaves, where conditions are seemingly more favorable for a rapid collapse of the leaf tissue than is the case on the younger leaves.

Considerable uncertainty has been experienced throughout the progress of this investigation as to the continued pathogenicity of the organisms carried in culture on potato-dextrose agar, and as a result frequent inoculations have been made to test this point with various cultures. In large measure, the same has been true of the wildfire organisms carried along simultaneously.

A large number of subcultures have died, lost their pathogenicity completely, or in considerable part (Pl. 2, B).

This has been due apparently in most cases to an unfavorable cultural medium, although in many cases this occurred on potato-dextrose agar made according to the same formula as other batches in which organisms have been kept alive and virulent through transfers kept in the refrigerator for three years or more.

A limited number of inoculations have been made upon other host plants aside from ordinary tobacco (*Nicotiana tabacum*). Infection has been secured upon various other species of Nicotiana, especially *N. glauca* and *N. rustica*, and also upon the tomato, together with some indication of infection upon certain cereals.

CULTURAL CHARACTERS

MORPHOLOGY.—The organism is a short motile rod with rounded ends, occurring singly, in pairs, or occassionally in short chains. Measurements under various conditions have ranged from 0.5 to 0.8 microns in width by 1 to 2.4 microns in length, averaging about 0.6 by 1.8.

Stained by the Caesar-Gil and modified Pittfield methods for flagella from 24-hour-old cultures, the organism shows from one to several polar flagella, usually two or three, but as many as seven have been counted. The flagella are ordinarily from three to five times as long as the organism itself. The organism has been stained with carbol fuchsin, methylene blue, and anilin gentian violet. No spores or involution forms have been observed. Capsules are formed. Pseudozoogloeae are apparently absent. It is Gram-negative and is not acid-fast.

POTATO-DEXTROSE AGAR.—Most of the cultural work has been done on potato-dextrose agar, as this had been found to be most useful for rapid comparative purposes on account of the color imparted to the agar. Colonies in agar plates are first visible in about 36 hours at about 22° to 26° C., increasing to 3 to 5 mm. in diameter in six days. Colonies are at first grayish white, changing on most media on about the third day to a distinct yellow, after which a transparent light yellow tinge develops in the potato agar. The colonies are round, shining, convex, and yellow with opaque centers. The submerged colonies are lenticular. On agar slants a distinct growth may appear along the line of inoculation in 24 hours, and this broadens gradually at the base, becoming echinulate (Pl. 4 A). The growth becomes fairly heavy in 3 to 5 days but rarely covers the slant, usually developing a fairly deep orange color. Certain potato-dextrose agar media will color up in a few days, but ordinarily more gradual coloration occurs, the media becoming usually a bright honey yellow, which may extend to a depth of 2 inches or more from the growth down into the tube. Where the pigment is rapidly absorbed by the agar, the growth does not take on a deep color. Most of the yellow bacterial plant pathogenes known to science have been cultivated simultaneously, but with none has this charactristic of yellowing potato-dextrose agar been nearly as distinct, and with the majority seemingly it does not occur. The pigment on potato-dextrose agar is soluble in water, sulphurous acid, ammonia, methyl and ethyl alcohols, and hydrogen peroxid. It is apparently destroyed by hydrochloric acid, tolulene, xylol, benzol, and carbon disulphid. Its production is slowed up markedly at 10° C., but between 20° and 33° it is normal. Light apparently does not influence its production. Its intensity varies considerably on different potato agars, which may possibly be due to differences in reaction of the media.

GELATIN.—Gelatin is rapidly liquefied in thickly sown plates, usually within 48 hours. In gelatin stabs liquefaction usually begins in 48 hours (assumes a stratiform shape), and may require two to three weeks for completion.

NUTRIENT BROTH.—Decided clouding occurs in 24 hours in beef-peptone broth (+10 Fuller's scale) with moderate flaky sediment. On slight shaking the sediment readily breaks up into a fine suspension. Cloudiness does not appreciably increase after 3 days. A very thin surface membrane may be formed, after several days, but this characteristic is not marked.

POTATO CYLINDERS.—Good growth in 48 hours, of brownish yellow color. Growth is profuse in 5 days, with increasing yellowing of organism along line of streak and darkening of medium. Tests with iodin indicate marked conversion of starch to amylodextrin, but diastatic action on the whole is feeble as compared with *Bacterium campestre*.

LITMUS MILK.—The color becomes more intense on the second day, with the formation of a thin blue layer in the upper portion of the medium which disappears about the third day. After 4 days the casein is precipitated, and in 6 more days the clearing has proceeded two-thirds of the way down the tube. The liquid is dark green on top and shades down to a tan just above the casein. The medium on long standing finally becomes a deep blue-green.

COHN'S SOLUTION.—Marked clouding occurs in 2 days or less, followed in about 4 days by the appearance of a heavy white crystalline membrane on the surface and a faint greenish tinge below it. On long standing the medium becomes light yellow in color and contains a flocculent precipitate.

FERMI'S SOLUTION.—Clouding becomes very marked after 2 days. After about 8 days the medium takes on a light greenish tinge, but this is not as marked as with *Bacterium tabacum*. After about 10 days or more a fairly heavy membrane is formed and the sediment increases. On longer standing the medium turns to an intense honey-yellow color.

BEEF AGAR STROKE.—On beef agar slants growth is distinct in 24 hours, grayish white in color, turning finally to a deeper yellow. Growth less profuse than on potato agar and no coloring of medium evident.

LOEFFLER'S BLOOD SERUM.—Growth grayish yellow, spreading, resulting in gradual liquefaction of medium.

NITRATE IN NITRATE BROTH.—There was no reduction of nitrates in nitrate peptone broth.

INDOL, AMMONIA, AND HYDROGEN SULPHID.—Negative tests for all by usual methods.

FERMENTATION TESTS.—From a 2 per cent Difco peptone solution five different carbon media were made by adding 1 per cent of the following: Saccharose, dextrose, lactose, glycerine, and dextrin. In fermentation tubes no gas was formed with any of these compounds. Distinct clouding appeared in the open arms in 48 hours. In the case of saccharose and dextrose slow clouding also appeared in the closed arms. No acid was produced in any of the tubes.

LITMUS SUGAR AGARS.—Tests with lactose, glycerine, saccharose, dextrose, dextrin, agars showed no formation of acid in any case.

TOLERATION OF ACIDS AND SODIUM CHLORID.—No growth was obtained in tubes of neutral beef-peptone broth to which 0.3 per cent of malic, citric, or tartaric acid had been added (P_H values 3.6 to 4.0). A concentration of 0.2 per cent of malic and tartaric limited growth but 0.2 citric did not. Two to 3 per cent sodium chlorid limited growth markedly and 4 per cent inhibited growth entirely.

OPTIMUM REACTION AND TOLERATION LIMITS.—The best growth in beef-peptone broth was secured at + 10 to + 15 Fuller's scale. The maximum for growth lies apparently close to + 20, + 22 giving no growth. While good growth was secured in some instances as low as −20 it is not believed that these results are significant, since the broth after adjustment and standing for some time usually rose to −4 or higher.

TEMPERATURE RELATIONS.—The optimum for growth in culture lies close to 26° to 28° C. No growth was secured at 35° to 36° or below 7° to 9°. The thermal death point found by subjecting freshly inoculated tubes of boullion to different temperatures for 10 minutes was found to be about 57°.

RESISTANCE TO DESICCATION.—The organism dried on cover slips in sterile Petri dishes did not lose its power of growth until after 14 days.

RELATION TO OXYGEN.—No growth in atmosphere freed from oxygen by pyrogallol-KOH method. Some growth in closed arm of fermentation tubes with saccarose and dextrose.

EFFECT OF SUNLIGHT.—Fifteen minutes' exposure of plates on ice to sunlight killed a few organisms, and practically all were killed on 30 to 60 minutes' exposure.

VITALITY AND VIRULENCE.—The organism can be kept in culture and maintain its virulence for at least three years. It may lose its virulence, however, upon certain media while still giving normal growth in culture or it may die out rapidly on presumably favorable cultural media for reasons not definitely understood.

GROUP NUMBER.—221.3333633. The name *Bacterium melleum*, n. sp., is suggested for this organism, basing the specific name on the honeylike color imparted to potato dextrose agar.

TECHNICAL DESCRIPTION

Bacterium melleum, n. sp.[3]

A short rod with rounded ends, occurring singly, in pairs, or in chains. Average size 0.6 by 1.8 microns. Motile by a tuft of polar flagella usually two to three in number, but ranging from one to seven, and three to five times as long as the body of the organism. No spores or involution forms have been observed. Capsules are present. It is Gram-negative and not acid-fast. The organism is pale or orange yellow on most media, particularly on potato-dextrose agar, to which it imparts a honeylike pigment. Growth on potatoe agar stroke is abundant, echinulate, raised, glistening, smooth, and viscid; agar colonies grow rapidly, are circular, smooth, and convex. On nutrient broth the surface growth is slight or none, clouding strong, and sediment somewhat flaky, more amorphous, and moderate in amount. In gelatin stabs growth is best at top with liquefaction, beginning in 3 days and complete in about 20 days. The coagulation of milk is prompt, coagulum slowly peptonized. Alkaline reaction with litmus milk, with prompt reduction. Good growth in Fermi's and Cohn's solution. No indol or ammonia produced. Nitrate in nitrate broth not reduced. Optimum temperature for growth about 26° to 28° C., maximum 35° to 36°. Thermal death point 57°. Optimum reaction for growth in broth +10 Fuller's scale. Pathogenic on *Nicotiana tabacum*, causing a leafspot with or without a chlorotic halo around the point of infection, usually followed by browning of affected tissue.

COMPARISON WITH OTHER BACTERIAL LEAFSPOTS OF TOBACCO

It is evident from the description of the causal organism that the Wisconsin leafspot differs from that of the previously described American tobacco leafspots, namely, wildfire and angular leafspot. The most striking difference is that of the color of the pathogenes, which is white in both of the latter diseases but yellow in the Wisconsin leafspot. As far as symptoms themselves are concerned, one could not with certainty distinguish between wildfire and Wisconsin leafspot, though the former is the more destructive and widespread and under field conditions usually possesses the most marked chlorotic halo.

The Wisconsin leafspot disease is not so readily distinguishable from the Sumatran disease described as black rust by Honing (*4*). Neither specimens of this disease nor the causal organism have been seen. Therefore Honing's description offers the only basis for comparison.

[3] According to the classification of the American Society of Bacteriologists the combination would be *Pseudomonas mellea* n. sp.

The illustration of black rust by Honing shows some resemblance to Wisconsin leafspot, but the disease does not seem to be identical with the latter. The chief points of difference between the two causal organisms may be summarized as follows:

Bacterium pseudozoogloeae (Honing)	*Bacterium melleum*, n. sp.
1. Produces "black rust."	1. Produces "brown rust."
2. Apparently no chlorotic halo.	2. Chlorotic halo frequently present.
3. Produces lesions with concentric rings.	3. Lesions usually not concentrically ringed.
4. Size generally 1.5 microns by 0.7 to 1 micron.	4. Generally 1.8 microns by 0.6 micron.
5. 1 to 2 polar flagella.	5. 1 to 7 polar flagella.
6. Color usually yellowish gray.	6. Color usually orange-yellow.
7. Gelatin stab papillate; liquefaction napiform to saccate.	7. Gelatin stab filiform; liquefaction stratiform.
8. Milk coagulum not peptonized.	8. Milk coagulum peptonized.
9. Litmus milk rendered acid.	9. Litmus milk rendered alkaline.
10. Acid with dextrose, lactose, and saccharose broth.	10. No acid with dextrose, lactose, and saccharose broth.
11. Fluorescence yellowish green (in gelatin).	11. Fluorescence honey-yellow on potato-dextrose agar.

From this comparison it may be seen that the differences are decided in many instances and that the likelihood of the Sumatran and American leafspot being identical is very remote.

PRACTICAL CONSIDERATIONS

The Wisconsin bacterial leafspot or "rust" no doubt occurs annually in this State, or at least it has been found for the last five years without much difficulty, although not to such an extent as to cause much concern. As already stated, however, it is quite certain that in years past it has been the cause of considerable losses and the object of demand for control measures. This belief is strengthened by the writer's recent studies of nonparasitic spotting of tobacco, which might otherwise have been confused with the bacterial leafrust. The causal organism is not believed to be a vigorous parasite, and special conditions are necessary for infection without wounding. Aside from a period of rainy or humid weather, and possibly a fairly high temperature, we do not know the conditions which are necessary for infection, since these two requirements in themselves are apparently not sufficient. This conclusion is arrived at as the result of environmental studies in controlled temperature and humidity chambers.

There is some ground for the belief that plants may be predisposed to the disease from internal causes. This hypothesis may be illustrated by an observation of the behavior of this disease in field fertilizer plots at the Wisconsin Experiment Station. The plots concerned were in duplicate and were intended to compare the value of barnyard manure with commercial fertilizers. For some reason not clearly understood, the manured plots gave a yield considerably lower than the unfertilized plots. Counts were made of the number of infected plants in each plot and, as may be seen from Table I, a fairly close correlation existed between yield, or the fertilizer applied, and the amount of infection.

TABLE I.—*Percentage of "rust" on fertilizer test plots*

Plot No.	Fertilizer applied per acre.	Yield in pounds of cured leaf per acre.	Percentage of plants rusted.
3	Barnyard manure, 20 tons	1,545	43
8	do	1,242	46
	Average	1,393	44.5
1	No fertilizer	1,682	23
7	do	1,527	26
	Average	1,604	24.5
2	200 pounds nitrate of soda, 200 pounds sulphate of potash, 600 pounds acid phosphate.	1,695	11
6	do	1,735	10
	Average	1,715	10.5

It does not follow from this, however, that "rust" is more likely to occur in low-yielding crops, the manured plots in this test being in fact an average crop. The condition is rather one of "physiological or nutritional balance" in the plant, a condition, as yet very inadequately understood in relation to plant diseases. A practical suggestion for experimental work in reducing damage from this disease by proper fertilization is offered, however, by such observations.

It has usually been found that the first or primary infection starts in the seed bed and that the secondary infection in the field is a direct result of transplanting infected plants. The same seems to be especially true of the wildfire disease. In the springs of 1917 and 1918 infection with Wisconsin leafspot was first noted in the seed beds at the Wisconsin Agricultural Experiment Station, and subsequently secondary infection in the field was found in areas in the field planted from the infected areas in the seed beds, although a considerable time intervened between the two appearances of the disease, during which time it had apparently disappeared. This experience, together with a similar, and now common, experience with the wildfire disease, offers the suggestion that seedling plants from areas showing signs of infection should preferably not be transplanted into the field. It is not improbable that spraying with copper sprays in the seed beds as suggested for wildfire (5) may also help to control the Wisconsin bacterial leafspot should conditions warrant its use.

The manner in which the disease lives over winter is not definitely known. The causal organism may perhaps live over on the seed, on the cloth covers, possibly in the soil, or by other means. Until this is determined no satisfactory means of control from this standpoint can be offered.

Although a considerable number of varieties of tobacco have been grown at the Wisconsin station annually, these have not all been equally exposed to infection. From limited observations and experiments, however, the writer feels safe in concluding that differences in varietal susceptibility or resistance are small, if in fact they exist at all.

SUMMARY

A bacterial leafspot disease of tobacco has been under observation and study in Wisconsin for five years. This disease is one of three or more different leafspots of tobacco commonly grouped under the common name "rust" by the growers in Wisconsin. The other "rust" spots appear to be nonparasitic in nature.

This disease has not been especially serious in recent years, but it is believed that it is the "rust" which has been most serious in past years and may become so again at any time that the required favorable conditions for its occurrence appear.

The Wisconsin leafspot of tobacco differs from both the wildfire leafspot and the angular leafspot occurring in other sections of the United States, and from the black rust occurring in Sumatra, all of which are bacterial in nature. The symptoms of the wildfire disease and of the Wisconsin leafspot, however, are much the same.

The disease usually manifests itself by round, brown, or rust-colored spots, usually less than ½ inch in diameter, but frequently running together to form larger irregular lesions. Frequently the young lesions are marked by a distinct chlorotic area or halo surrounding the point of infection. Under field conditions infection usually starts on or is confined to the lower leaves. Lesions may also occur on young leaves in the seed beds.

The disease is caused by a yellow bacterial organism apparently previously undescribed. The name *Bacterium melleum*, n. sp., is suggested, and the more common morphological and cultural characteristics are given.

Artificial infection has been secured only through wounding by needle pricks. Under the conditions of the inoculation experiments in the greenhouse this has also been more or less true with the wildfire organism, which has been studied comparatively in practically all of the work done with the Wisconsin leafspot organism. Under field conditions it is not believed that wounding is necessary for infection. Temperature and humidity conditions in themselves do not apparently govern the occurrence of infection. Some data secured indicate that predisposition may be influenced by the fertilizing materials available.

It is believed that the disease ordinarily starts in the seed beds, from which it is transferred to the field. Growers are advised, therefore, not to use plants from infected seed bed areas for transplanting.

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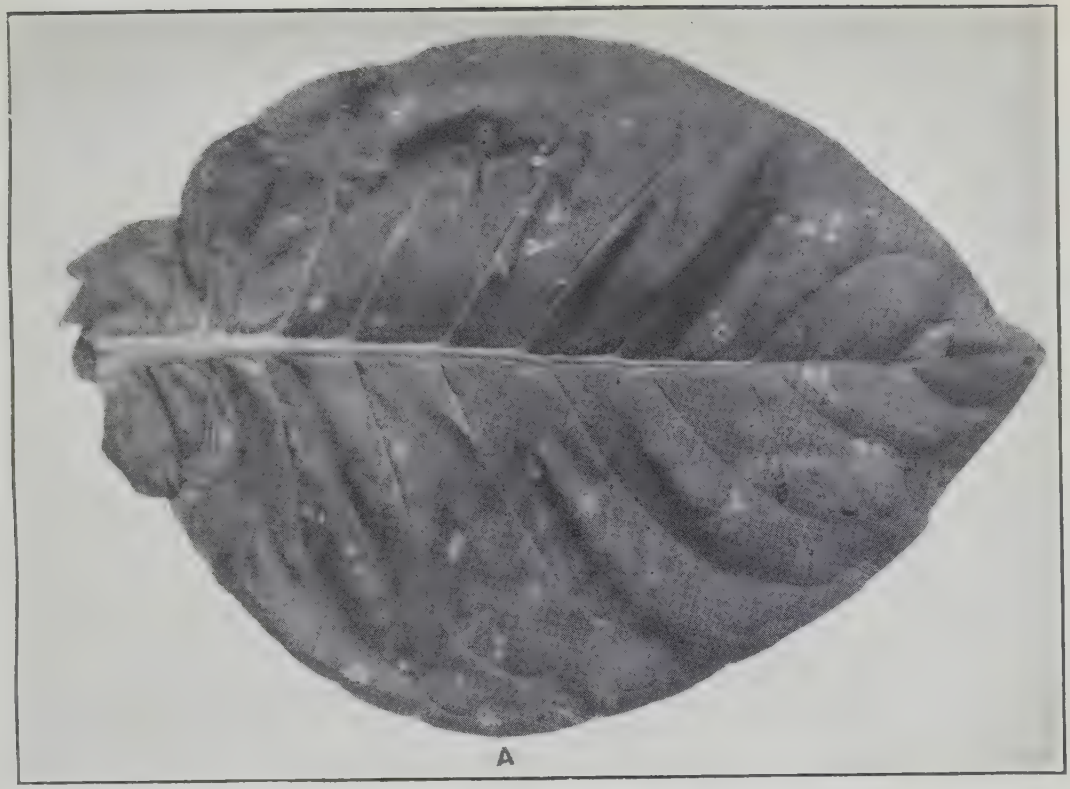
PLATE 1

A.—Leaf of Havana Seed tobacco, showing fairly typical symptoms of the Wisconsin leafspot ("rust") disease. Natural infection.

B.—More detailed view of portion of leaf similar to that illustrated in A, showing the central points of infection, the coalescence of the spots, and their relation to the veins.

C.—A leaf showing a fairly typical case of "wildfire" on Havana Seed tobacco. Compare with Wisconsin leafspot. This leaf was collected in Connecticut in July, 1919, and was the first authentic case of the wildfire disease in New England.



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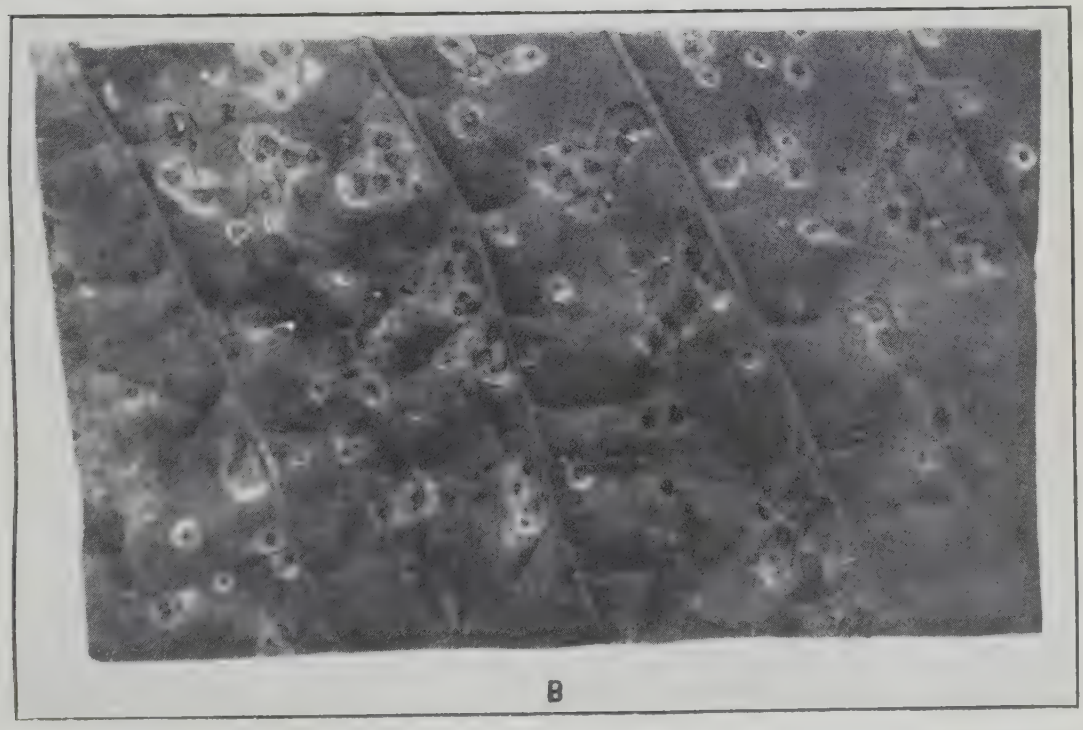

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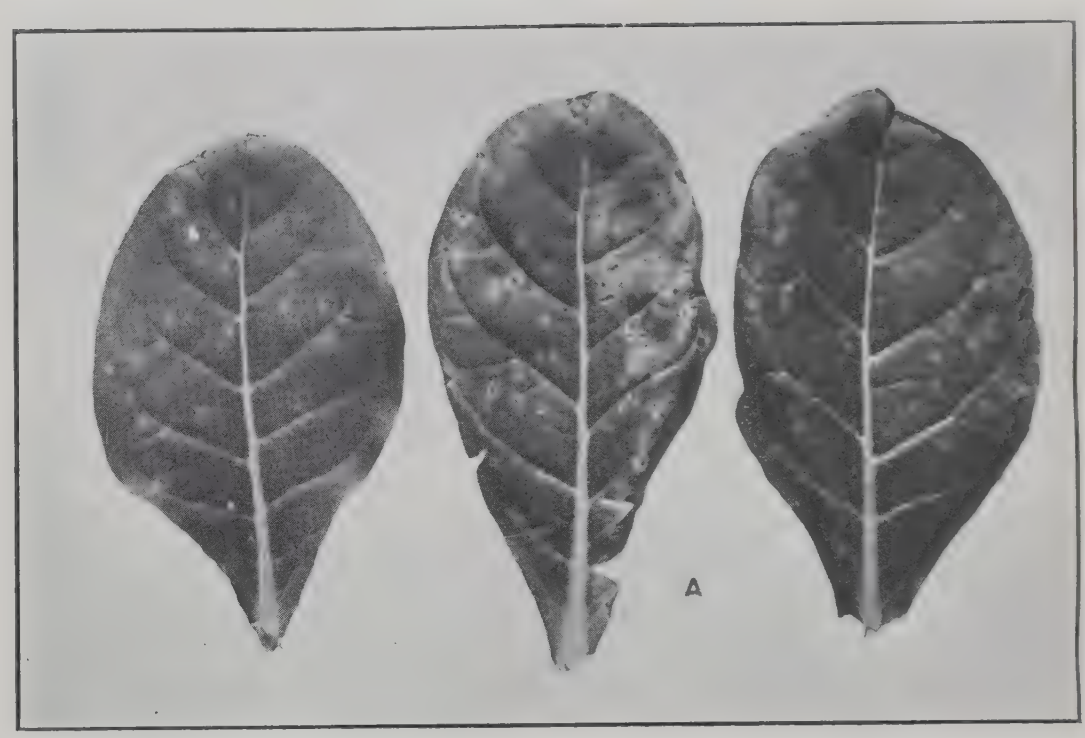
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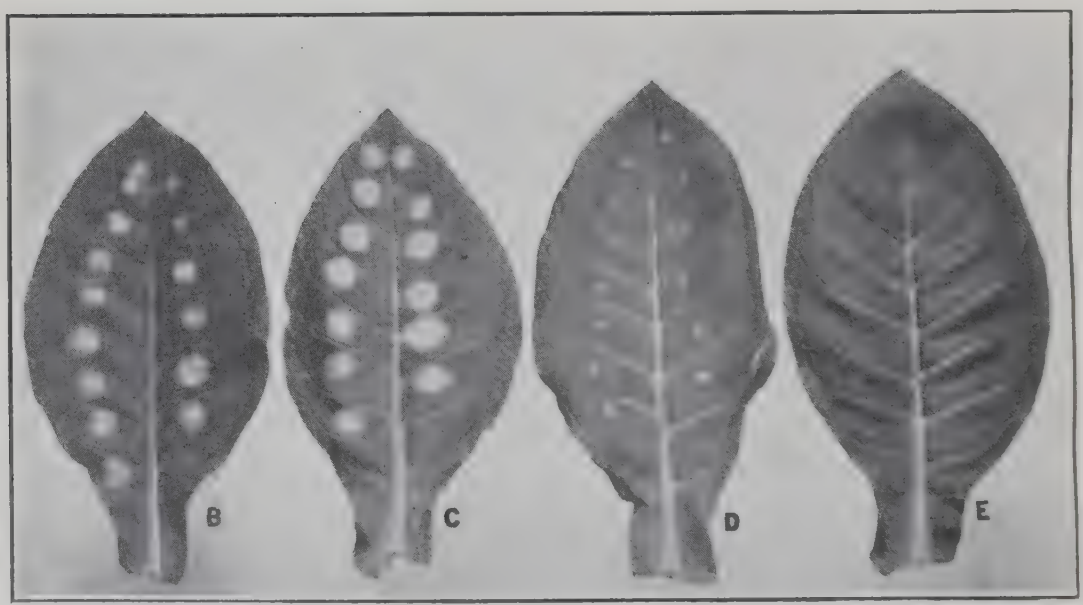
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PLATE 2

A.—Leaves from tobacco seedlings in seed beds, showing typical lesions. Such leaves readily carry the infestation to the fields. Note the halo surrounding the points of infection (B, C, and D). Artificial inoculation with leafspot organisms by means of pin pricks on tobacco leaves, illustrating their similarity under certain conditions (B, C, D, and E).

B.—Leaf inoculated with Wisconsin leafspot organism.

C.—Leaf inoculated with wildfire organisms.

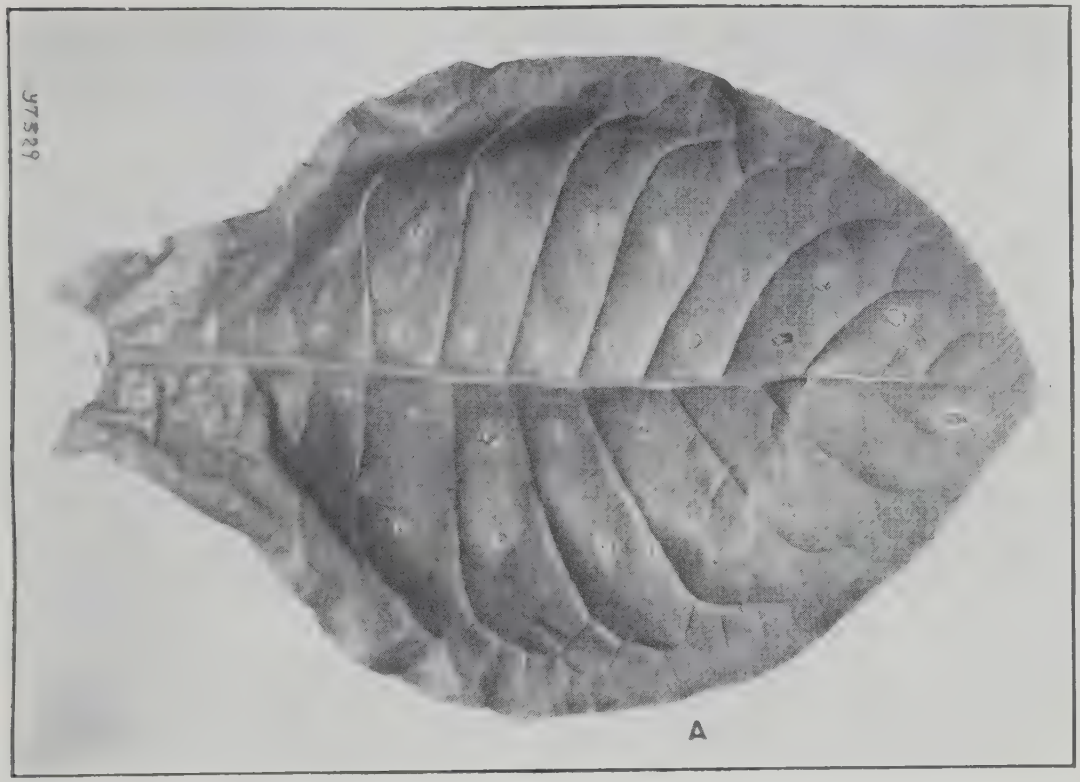
D.—Leaf inoculated with Wisconsin leafspot organism with lowered virulence.

E.—Control, pricked, but not inoculated.

PLATE 3

A.—A leaf of tobacco inoculated with the Wisconsin leafspot organism by means of pin pricks. Tissue collapsed rapidly around point of inoculation and turned brown, no halo developing. Similar spotting without halo may also develop in the wildfire disease.

B.—Typical colonies of the Wisconsin leafspot organism on potato agar plate.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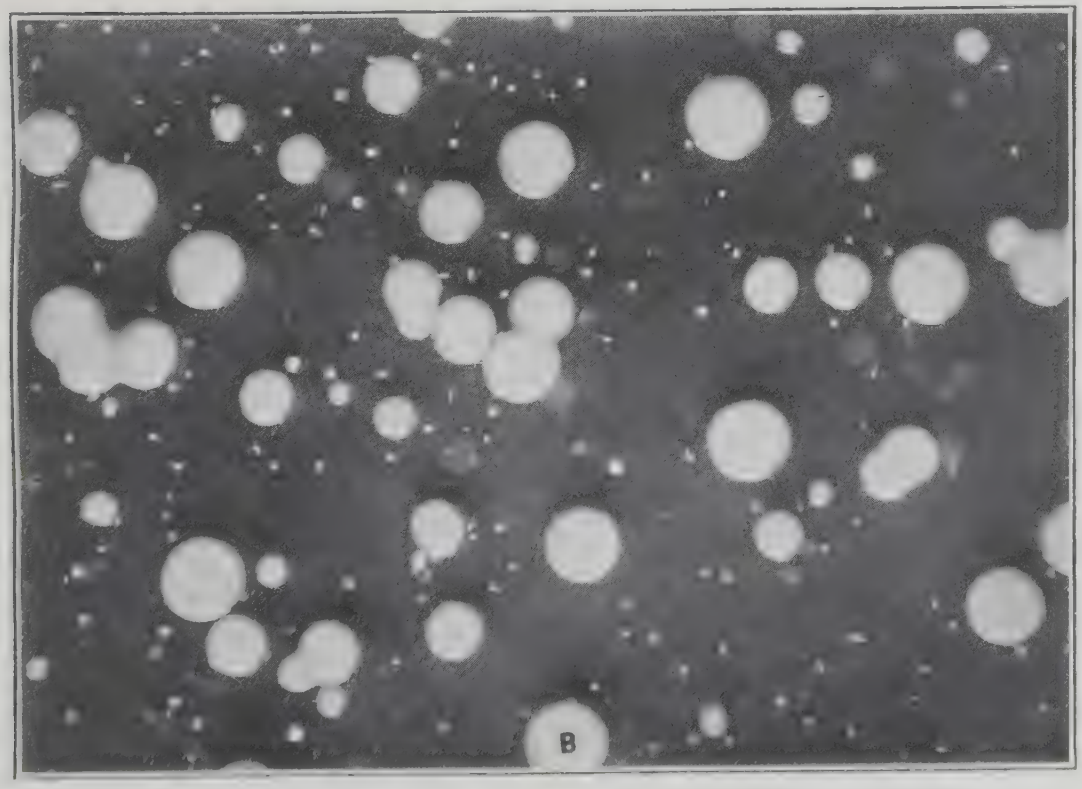
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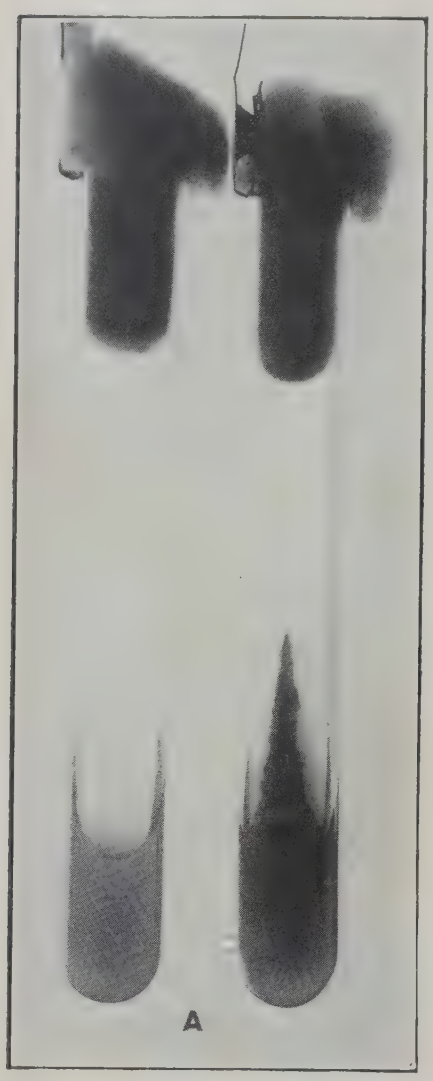
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PLATE 4

A.—Streak of Wisconsin leafspot organism on potato-dextrose agar after four days' growth at 20° to 22° C. in comparison with control tube. Note color difference of culture medium, as a result of development of yellow pigment on potato-dextrose agar.

B.—Photomicrograph of Wisconsin leafspot organism. Carbolfuchsin stain. ×3,000.

C.—Line drawing of Wisconsin leafspot organism, showing flagella.

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PARASITIC FUNGI INTERNAL OF SEED CORN [1]

By THOMAS F. MANNS, *Pathologist and Soil Bacteriologist*, and J. F. ADAMS, *Associate Pathologist, Department of Plant Pathology and Soil Bacteriology, University of Delaware*

The importance of root, stalk, and ear rot fungi in decreasing yields of corn has received considerable attention in recent years on the part of investigators. Results of investigations so far reported indicate more or less agreement in the various disease symptoms manifested. However, some difference of opinion exists concerning the importance of the causal organisms. The specific determination of the fungi has not been fully emphasized nor the method by which they are carried in the seed.

The following report presents in part the results of our investigations in determining the species of fungi associated with corn rots. Our studies were initiated to ascertain the losses to the corn crop and prevalence of infection in Delaware and the importance of the seed in carrying infection. While our observations and studies have been confined principally to field corn in this State, we feel that careful investigations will reveal the presence and importance of the same pathogenes in other States but varying somewhat in prevalence.

A review of the literature covers the observations and investigations conducted in this country so far as we have been able to secure them. In this review only special mention is made of results bearing directly on seed infection, the fungi reported, and the method by which the fungi are carried in the seed. Because of the extensive observations reported on the root, seedling, and stalk infection it was deemed best to discuss this phase of the problem in a subsequent paper to be prepared by the writers.

"Moldy corn" no doubt was one of the first symptoms observed in connection with corn rot diseases. The presence of mold in corn was early associated with forage poisoning and pellagra in this country, as well as in Europe. Starting with these early observations were studies which were later extended to include and establish the importance of the fungi which are now associated with the corn rots.

Sheldon (*29*)[2] described *Fusarium moniliforme* Sheldon, associated with moldy corn and thought to be the cause of forage poisoning. He observed—

> the kernels where the pink mould was present were considerably broken at the ends and crumbly so that when shaken or struck on the table, the inside would fall out in

[1] Accepted for publication July 18, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 523–524.

the form of a coarse powder; it attacks the end of the kernel first, gradually working its way toward the cob.

Valleau (*36*) in his studies of corn rots in Kentucky as well as those from other States, considers that *Fusarium moniliforme* will probably prove to be the most common cause of root and stalk rots of corn. He finds that—

a microscropical examination of the pink crowns or pink stripes on kernels reveals the presence of hyphae and occasionally spores in them, between the seed coats. The development of reddish or black discoloration in the seed coats of corn during and after germination, is an indication of infection with *F. moniliforme*.

No mention is made of the importance or determination of other fungi in connection with his studies. In a later article (*37*) on the control of corn root rot, it is stated that—

thus far no disease free seed has been obtained by the method of selection. The results obtained indicate that infection takes place before the early dough stage. It probably occurs through the silks and is a result of infection of the exposed silk mass with *F. moniliforme*. Attempts to control corn rot by seed treatment have given negative results.

Barrett (*5*) reported on the dry rot in corn as caused by several species of fungi. One of the most destructive being *Diplodia maydis* Sacc. (*D. zeae* (Schw.) Lev.). A second form of dryrot which is not uncommon is due to a species of Fusarium, and a third form is due to a sterile fungus. Later in conjunction with Burrill (*6*) studies were reported on ear rots of corn. Four types of ear rots were recognized as caused by *D. maydis* and three different fusariums, designated 1, 2, and 3.

The most extensive work was on *Diplodia zeae*. They observed—

the slender threads penetrate the young tissue of the grains, cob and husks, progressing from cell to cell and extracting from their contents whatever is of value for food. Diseased ears left in the field under natural conditions eventually develop numerous pycnidia in the grains, giving them a black appearance.

The best results of inoculations were obtained when the corn was still in the thick milk stage.

80% of silk inoculated ears produced the disease, 71.7% of those inoculated at the base and 48% so treated in the shanks were successful, while but 22% of the sprayed ears showed any signs of infection. All later inoculations altho fairly successful produced smaller percents of the disease than others.

There was no direct evidence obtained that the infected ears on inoculated stalks were a result of the inoculations made.

Fusarium 1—

produces a rather dense, felty mass of white mycelium which extends between the kernels to the cob, causing it to become more or less diseased.

The mycelium is established all through the diseased grains, corroding the starch and destroying the germs.

The diseased portion of ears infected with Fusarium 2 have a deep pink to red color due to the pigment produced in the hyphae of the fungus.

The felty mass of mycelium permeates the inner husks and silk and holds them firmly to the ear.

Fusarium 3 forms a rot less complete in its destructiveness of the ear than that of the other forms described. Many of the infected ears have only a few scattered disease grains.

Under some conditions, however, most of the kernels may become diseased and the cob more or less infected . . . . The mycelium is white, very sparse, and is

found principally in the end of the kernels where it feeds upon the starch and produces large numbers of spores, mostly microconidia.

Corn inoculated in the thick milk stage shows the largest percentage of infected ears in the field.

It is worthy of remark that in no case has natural infection by these parasites been discovered upon any other part of the immature corn plant save the ears and their belongings. Upon the latter infection always begins externally from air distributed spores.

The parasitism of *Diplodia zeae* has been more thoroughly established than any of the other fungi associated with corn rots. Heald, Wilcox, and Pool (*16*) state that this fungus produces in the ear a condition which may be called "dry rot."

In the early stages or in cases of slight attack, no external evidence of the presence of the fungus can be detected. It is probable that the hyphae enter the kernel at its base, since the fungus is first detected in this region. From this point the hyphae grows throughout the kernel and are found in both the endosperm and embryo.

They consider the prevailing type of infection is through the silk near the time of pollination. This disease was observed by Stevens and Hall (*33*) in North Carolina where it is known as "mold," "mildew," "rot," and "souring."

The disease affects the ear, manifesting itself as a whitish growth of mold over the surface of the grains, sometimes affecting the whole ear and at other times portions only of it. The amount of fungus visible upon the superficial parts of the grain is not large, but upon breaking open the ear, it is found that the spaces between the bases of the kernels are often densely packed with masses of pure white mycelium. Externally, no signs of fruiting bodies of any kind are apparent, but close examination at the point of attachment of the grains to the cob, in many instances, reveals the presence of exceedingly minute black specs, which under the microscope prove to be the fruiting organs (pycnidia) of the fungus.

They determined the fungus as *Diplodia macrospora* Earle, but *Diplodia zeae* Lev. also was found in several instances. Smith and Hedges (*31*) concluded on the basis of pot experiments that the manner of infection indicated is the common one, that is—

from the soil into the roots, from these to the interior of the stems and thence upward to the cobs, and finally to the kernels.

Evans (*11*) reports maize cob mold (*Diplodia maydis Sacc.*) as prevalent in Natal. It is also frequently referred to as mildew.

Many of the farmers showed me samples of mildewed maize cobs which they stated caused them severe loss in their crops, and also produced severe paralysis and frequently death amongst stock that were fed on these cobs, especially if the cobs were damp and not properly dried out.

Van Der Bijl (*38*) conducted extensive studies on the "Dry-Rot" of maize in South Africa and considers that normally infection takes place through the silks. A Fusarium is also mentioned as being found several times with moldy corn. Garman (*12*) states that—

Diplodia zeae is not dependent on the openings made by the worm, generally invading ears by way of the shank.

Fusarium-like fungi for the greater part were associated with these corn diseases by the early investigators. It was not until recently that several recognized species of Fusarium were definitely established with the root, stalk, and ear rots of corn.

Garman (*12*) along with his studies of pellagra mentions Fusarium and *Tricothecium roseum* associated with moldy corn. He states that—

seed corn should be inspected for molds with special care, a pink mold (a *Fusarium*) is very common in our fields and causes many grains apparently sound to assume a

pink color. What appears to be the same fungus is on corn that is germinating badly in the field.

Arzberger (*4*) investigated the cob rot of corn caused by *Coniosporium gecevi* Bubák. It is reported that—

Coniosporium has an economic significance in that it destroys the cob tissue as a saprophyte; its effect on the kernels is rather limited when compared with the injury of *Diplodia*, *Fusarium*, and other fungi.

Pammel (*24*) briefly described a serious root rot and stalk disease of corn and later with King (*25*) published their studies on a Fusarium disease of corn and sorghum.

This fusarium disease attacks the roots, the stalks, and the ears of corn at least some seasons. It has not been determined whether all the symptoms are caused by the same organism or not.

Under the subject heading "Ear Rot," it is stated that—

the molds are of three kinds. One attacks the kernels, husks, cobs, and sheaths, the threads of the mold occurring thru the cobs and sheaths, and destroying the kernels completely. The second kind of mold produces a deep pink or red color. The threads of this fungus are felty and penetrate the husks. The kernel becomes brittle and red. The third type of mold attacks an occasional kernel and is not so serious. Its threads are white. A fourth fungus,parasitic in character, was found on the ears, stems, and sheaths, and also on the roots. It is known as Diplodia Rot.

Regarding the Fusarium—

the mycelium penetrates not only the living cells, but also occurs in the intercellular spaces. It occurs abundantly in the embryo and endosperm of the seed. The inoculation experiments indicate that the fungus enters not only with the seed, by seminal infection, but that the undeveloped shoots in the axils of the leaves probably are responsible for some of the infection in the field. The disease probably spreads largely with the seed corn.

No determination of the Fusarium was made by the authors. They observed the perithecia of Gibberella abundantly on the sheaths and stem of corn plants but were unable to show that this fungus is connected with the Fusarium studied.

Hoffer and Holbert (*18*) in 1918 first published on their extensive studies of the root, stalk, and ear rot diseases of corn. It was stated that—

inconspicuous rotting of the stalks, of the ears, and of the roots, may take place with no apparent injury. The kernels from ears borne on diseased plants will have seedling characteristics which can be noted usually on the germinator. Those seedlings which have rotted embryos and stalks indicate the ears to be discarded for seed purposes. The harmful organisms referred to in this bulletin are species of *Gibberella*, *Fusarium*, *Verticillium*, *Rhizopus*, and *Pseudomonas*.

No determination of the species was mentioned at this time. In a later publication (*20*) it is reported that—

these rot diseases are caused by a number of factors working more or less together, some of which are well known and others less fully known. Investigations have shown that certain fungi *G. acervalis* (Moug) Wr. and *G. saubinetii* (Mont.) Sacc. as well as certain bacteria are commonly present in diseased corn plants in the field. On the germinator those organisms as well as certain molds (*Rhizopus*, *Aspergillus*, etc.) also may occur on diseased, weak, or immature kernels and seedlings.

These authors in a recent abstract (*21*) on corn root and stalk rots consider the pathogenes are chiefly species of *Fusarium* and *Gibberella*.

The common wheat scab organism, *G. saubinetii* (Mont.) Sacc. is probably the most common pathogene responsible for much of the root and stalk rotting of corn plants in the Central States.

Hoffer (*17*) reports similar observations for sweet corn—

> It is believed that the greatest damage to large numbers of seed ears by harmful organisms occurs when the ears are left in the field for long periods after maturity.

Stover (*34*) in Ohio testing seed corn for germination mentions the presence of Fusarium-like fungi. Gilman (*14*) reports a Fusarium-wilt of corn in Iowa. Isolations established the presence of a Fusarium in 93 per cent of the cases with seedlings 6 inches high showing a brown discoloration of the vascular system at the crown.

The literature as reviewed establishes the fact that exclusive of Diplodia, only species of Fusarium have been definitely established with the rot diseases of corn. Germination tests have been observed to reveal the presence of the fungi, but no studies have shown the method by which the pathogenes are carried in what appears to be healthy, normal seed corn.

METHODS

The manner in which infection is carried in seeds showing no external symptoms was determined by germination, cultural, and histological studies.

A representative sample of over 100 kernels was selected from each ear of corn, 10 of which were used for each germination. The germination test was conducted for the most part in a Geneva germinator and with a modified rag-doll type of germinator. This method of germination gives complete evidence as to the relative germination or viability. Such a test does not provide in all cases for an accurate determination of what fungi are being carried internal of the seed. The seed from harvesting to the usual time of germination is exposed to contamination by many saprophytic and in some instances parasitic fungi. The growth from such superficial adhering spores may be so rapid and abundant as to make the identity of the internal parasites impossible. At the end of 7 or 10 days the seedlings were examined for internal symptoms as suggested by Hoffer and Holbert (*20*) in their studies. This point will be discussed in more detail in a later paper on seedling and stalk infection.

The most accurate test that we have found for determining the presence of fungi internal of seed corn, and one which at the same time readily permits of the identification of the fungi, is carried out by disinfecting and planting the kernels or crushed kernels in sterile culture medium in Petri dishes as shown in Plates 3, 4, and 11. Fifteen or more kernels are disinfected in a test tube 150 by 20 mm. for one minute in a solution of 50 per cent alcohol containing 1 gm. of bichlorid of mercury in each liter. This solution is known as a 1 to 1,000 $HgCl_2$ in 50 per cent alcohol. Following this treatment the kernels are washed in the same tube with two successive washings with 20 cc. each of sterile water, and immediately 10 kernels are removed aseptically with sterile forceps and placed with the germ side down on 20 cc. of nutrient dextrose agar in a sterile culture dish. Further, 5 of the remaining kernels are each placed in a sterile culture dish, and with a sterile scalpel the point of the kernel, which is the portion that contains most of the internal infection, is cut off $^1/_8$ to $^1/_5$ inch from the end; then with a strong sterile forcep each point is placed in the mouth of a heavy-walled tube (it requires a strong tube and strong forceps, as crushing is not easy) 150 by 20 mm., containing 10 cc. of sterile nutrient dextrose agar medium at 43° C.; the point is thoroughly crushed and shaken down into the medium,

then well mixed and poured into the sterile culture dish containing the remaining part of the kernel. These methods were used extensively by the senior author in his studies on fungi internal of Flax in 1904 [3] and wheat in 1909 (*28*). In this manner a greater distribution of the mycelium or spores is possible and allows for accurate interpretation in instances where more than one fungus is being carried. Some of the kernels carried three parasitic fungi (Pl. 5, 10, and 11); crushing was the most efficient means of determining all fungi present; on the plate containing uncrushed kernels the faster growing fungi inhibited the others. The plates were read at the end of 7 to 10 days for the presence of all bacterial and fungous growth.

In most of the cultural plate work a dextrose-peptone agar (tap water 1,000 cc., dextrose 10 gm., peptone 1 gm., agar 15 gm.) was of great value in differentiating the several parastic fungi. Twenty cubic centimeters of medium were used in all cultural plates in which 10 kernels of corn were placed for germination. For cultural work in further differentiating the various pathogenes potato starch, rice, and lima bean and sweet potato agar. were used as media. None of the media was standardized because the slight acidity resulting was favorable for differentiating in particular the various species of Fusarium.

Owing to the texture of the ripened kernel, histological methods of investigation meet with difficulties. The germ end and the cob, which are most important for studying, are very horny in texture and difficult to section. Fixation is not so difficult, and in our work Carnoy's killer was found most satisfactory for thorough penetration. If the kernels to be fixed are first cut in half, better killing and infiltration of paraffin is secured. The kernels are best softened for the killer by soaking for 24 hours in warm water, boiling till inhibition is complete, or steaming for 10 minutes in the autoclave. Even this treatment does not give satisfactory softening of the cap, which is the most difficult to cut. After killing, the kernels are dehydrated by the usual method, and serial sections are cut at 10 to 15μ in thickness on the microtome. All material was differentiated with Flemming's triple stain. Rotted kernels resulting from infection with *Diplodia*, *Gibberella*, and *Fusarium moniliforme* are easily prepared for histological studies by soaking eight hours in water.

CEPHALOSPORIUM [4] SACCHARI BUTLER

A fungus unlike any previously reported in this country as far, as we could determine, was found very prevalent internal of seed corn. This fungus morphologically agrees with the description of *Cephalosporium sacchari* Butler, as reported by Butler and Kahn (*8*) on sugar cane in India. Butler (*7*) also found this fungus on sugar canes shipped from the United States to India. In view of these facts and because of the close

[3] MANNS, Thomas F. FUNGI OF FLAX SICK SOIL AND FLAX SEED. 1904. Unpublished manuscript (Master's thesis) filed in Dept. of Botany, College of Agriculture, Fargo, N. Dak.

[4] In February, 1922 and again in April of the same year, Dr. E. J. Butler, of the Imperial Bureau of Mycology, Kew, England, kindly sent the authors cultures of *Cephalosporium sacchari* isolated from sugar cane by Dr. Shaw, of Pusa, India. There was some question in Dr. Butler's mind as to whether the first isolations were the same as the organism he originally described as *Cephalosporium sacchari*. In the cultures sent in April, 1922, Dr. Butler in examining them stated that "It seems to be pretty near the fungus as I know it." Both of these cultures in our hands proved to be a Fusarium, a fungus entirely different from the organism which we have tentatively referred to as *Cephalosporium sacchari* Butler. This Fusarium sent us by Dr. Butler when grown on nutrient dextrose agar gives a strong purple color. It is probable that the organism we have tentatively referred to the species *Cephalosporium sacchari* is entirely different; if the cultures Dr. Butler sent us are identical with the organism he described as *Cephalosporium sacchari*, then his species should become *Fusarium sacchari* (Butler). Our *Cephalosporium* must receive then further consideration. (This footnote was added February, 1923.)

relationship between the two hosts, it seems better to refer our fungus tentatively to this form than to create confusion by describing a new species. Further studies are in progress to determine the status of our fungus.

Butler and Khan describe the symptoms on half-grown canes as follows:

> At this period affected canes lag in growth, and stunted, single stools, or patches of varying size, may soon be observed scattered through the fields in which the disease is prevalent. From this on until the time of harvest withering of individual canes, or even of whole stools occurs. The leaves dry up, as if insufficiently supplied with water, followed by the stems which become light and hollow. If the cane be split longitudinally when the leaves are just observed to wither, a characteristic discoloration of the pith may be observed.

With the exception of a slight yellowing discoloration at the germ end, no external symptoms on seed corn has so far been observed which assist in determining the internal infection by this fungus. Physical characters of the cob, except slight discoloration, have given no indications. Shallow dents, dull appearance, loose cap of kernels have not been correlated with infection. In some instances the pith at the butts of ears is discolored and shredded, but this condition is not more associated with infection by this parasite than with that by any of the other species studied. The infection so far has been determined only in kernels that appear normal. The fungus has been found occasionally associated with the typical kernel rot produced by *Fusarium moniliforme*, but thus far we have not found it responsible for any specific symptom of disease on kernel. Germination of kernels does not appear to be seriously inhibited. On the agar plates the roots are often discolored about the seventh day. Inoculations at nodes and internodes in plants at various stages of development up to maturity has established the parasitic character of this fungus on corn. On the germinator the fungus grows out at the cap and develops a very modified white growth of mycelium. The mycelium is white and does not spread in growth like the other forms. In the limited growth numerous short white filaments are observed. These range from an $\frac{1}{8}$ to $\frac{1}{4}$ inch in height and are coremial-like growths (Pl. 10, G.). Infection with *F. moniliforme* may also be associated with this fungus in which case the more spreading and effused growth overruns and conceals the growth of *Cephalosporium sacchari*.

On culture media the growth is flat with little aerial mycelium produced. In some cultures, upright white coremial strands appear, as shown in Plate 10, G. The oppressed growth on the surface of sugar media as well as the spores en mass are salmon colored. On starch media a slight effused grayish growth is produced. Butler and Khan (8) give the following discussion of spores:

> Conidia borne on short, simple or branched, lateral hyphae and also terminally on the ultimate branches of the mycelium. They measure 4–12 μ (usually 5–8 μ) by 2–3 μ when formed, but increase in size prior to germination. Their shape varies from shortly oval to ovoid or long elliptical. Occasionally they are curved or with one side flattened. Some become septate prior to germination, the septa being 1–3 in number.

The conidia as described by these authors are aggregated into globules at the tip of conidiophores. They are typical glomerules, but the spores are not held together in a slime as is characteristic for the genera Hyalopus and Gliobotrys. We find the conidia on the host measure

3.5 to 8 μ by 1.8 to 2.6 μ. On culture media their range is somewhat longer, as 4.3 to 10 μ by 1.7 to 3 μ. The conidiophores range from 16 to 40 μ in length. The conidia at time of germination are much larger and often septate, as shown in Plate 1, B.

GIBBERELLA SAUBINETII[5] (MONT.) SACC

This parasite has been observed extensively on cereals in this country as well as abroad. Johnson, Dickson, and Johann (*22*) have shown this to be the most prevalent organism in producing wheat scab. In their study of over 1,000 specimens collected in 15 states, *Gibberella saubinetii* proved to be the chief causal organism. Hoffer and Holbert (*20*) mention its importance in connection with the root and stalk rot of corn. The description of Fusarium 2 by Burrill and Barrett (*6*) strongly indicates they were working with this species.

As with the preceding species, no uniform symptom is constantly associated with the infection of seed corn by this parasite. Plate 13, A, B, illustrates the extreme type of infection. We have consistently observed such infection confined to the tip of the ear. Such ears show a superficial growth of the mycelium between the rows of kernels which are found to be rotted throughout. In some instances the silk is matted with the mycelium. It is possible that such an association indicates that infection was established before maturity. The ear illustrated in Plate 13, A, was exposed to the changeable weather conditions long after the usual harvest time. Kernels from the tip of this ear were disinfected and crushed in a melted tube of agar for a poured plate. A pure growth of *Gibberella saubinetii* was recovered. Kernels from the butt of this ear treated in a similar manner showed no fungous growth.

The symptoms and effect in the kernels are very similar to that of wheat scab infection. White varieties of corn often show a pink discoloration of the kernels, but it is not so conspicuous on yellow varieties. On shelling the kernels from the infected end, a pinkish discoloration of the fruit cups is often revealed. This appearance is only evident where heavy infection occurs. The rotted kernels are very brittle. The starch in the endosperm is loose or readily crumbles. The embryo in such seeds is completely destroyed. All parts of the kernels under such conditions are invaded by the mycelium. The mycelium is intracellular and intercellular with the same resultant effect on the fruit as reported by Adams (*1*, *2*) for wheat scab infection.

Infection of seed corn is also found which to all external appearance is healthy. On the germinator such corn will show the effused growth of the fungus at the cap in about five days. Germination is retarded and in many instances inhibited on the germinator by the presence of this parasite. The perfect stage has developed on infected kernels that have been allowed to dry out on the germinator under conditions of room temperature. We have not observed the perithecia to develop in any of our cultures. Perithecia are commonly found fruiting at the nodes of old cornstalks in the field. The perfect stage has been found fruiting abundantly on kernels as well as at the butt and in the fruit cups on ears overwintering in the field.

[5] See footnote 4, p. 500.

It is a common observation that where wheat follows corn wheat scab is more prevalent. The ascospores from infected cornstalks, kernels, and cobs overwintering in the field are, no doubt, in part the source of the inoculum. The kernels on the terminal portion of ears appears to be as subject to infection as any of the kernels in wheat heads. This would appear to have been the method of infection of the ears shown in Plate 13, A, B.

Hoffer, Johnson, and Atanasoff (*19*) report *Gibberella saubinetii* from corn and wheat which was used for inoculation on wheat, saying that—

> the organisms from both sources have also been found to be similar morphologically. In view of the facts developed by this evidence, it seems certain that these are intercrop parasites which are of great importance in developing control measures for one of the rots of the root, stalk, and ear of corn and for scab of wheat.

With the usual abundant fruiting of *Gibberella saubinetii* on old cornstalks it is not difficult to secure pure cultures in order to determine the species isolated from the growth on germinating kernels. The fungus produces a very effused growth on the various media used. When the surface of the medium is completely covered by the mycelium a carmine red appearance is produced and the aerial mycelium becomes yellowish in color. Conidia which are 3-septate to 5-septate developed very sparingly in culture, and no true chlamdospores were found. The conidia observed in our cultures were for the greater part 5-septate. Measurements were made from conidia on dextrose agar and averaged 42 to 52.5 μ by 3.5 to 4.7 μ. A good detailed description of this species is given by Wollenweber (*40*).

FUSARIUM MONILIFORME SHELDON

This fungus, described by Sheldon (*29*) in 1904 was not referred to again until the association of this species with seedling-blight of conifers. Spaulding (*32*) mentions this species among some damping-off fungi on seedling conifers. Hartley, Merrill, and Rhoads (*15*) found it to be the most virulent of those tested on seedling conifers. As previously mentioned, Valleau (*36*) considers this fungus as the common cause of the root, stalk, and ear rot of corn. Norton and Chen (*23*) recently called attention to a new parasite of corn. They discussed the resemblance of their fungus to *Oospora verticilloides* Sacc. They consider their fungus is similar to *Fusarium moniliforme*, in so far as the type of catenulate conidia is concerned, but failed to find any fusiform or macroconidia as described by Sheldon (*29*). No mention of macroconidia since Sheldon's studies has been described by any investigators in their studies with this fungus. They are sparingly developed, which accounts for their not being commonly observed. It is of further interest that a species of Fusarium should possess catenulate conidia, as this does not agree with the characters of this form genus. Appel and Wollenweber (*3*) and Sherbakoff (*30*) do not consider catenulate conidia in their description of this form genus.

The fungus *Oospora verticilloides* described by Saccardo (*27*) on corn is no doubt identical with *Fusarium moniliforme*. The illustration of spores and their method of formation and measurement (*26*) are identical. Later Deckenbach (*9*, *10*) and Tiraboschi (*35*) observed this fungus on corn in connection with their studies on pellegra. Deckenbach (*9*) observed the violet and lavender color this fungus takes on under cul-

tural condition. He also reports finding a similar color on seed corn naturally sick with Oospora. This discoloration of the grains we have commonly observed with infected kernels overwintering under field conditions. Tiraboschi (*35*) gives more of a detailed study to this fungus, and his results and illustrations are very convincing. The confusion in the past regarding the identity of *Fusarium moniliforme* and *Oospora verticilloides* is no doubt the result of the macronconidia being overlooked.

The symptoms on seed corn produced by this fungus are numerous and more commonly found than those produced by any of the other internal parasites. The first conspicuous symptom is that of the typical kernel rot. Such infected kernels appear irregularly distributed in the ear as shown in Plate 12, D. This kernel rot in appearance agrees with the one described by Burrill and Barret (*6*) as caused by their Fusarium 3.

The condition of such infected kernels is similar to Sheldon's (*29*) description. The kernels appear slightly shrunken and light brown in color. Such kernels are soft and fragile, with the contents powdery, except for the embryos, which are discolored and shrunken. In some instances a slight development of mycelium and spores is found on the surface. Other symptoms of infected kernels are discolorations of the seed coat. These discolorations vary from a light brown to pink and lavender in color. They may be evident on any part of the kernel but are most commonly found near the germ end. This discoloration is the result of the development of mycelium between the integuments. These symptoms become more pronounced during germination because of the activity of the fungus. In some instances the fungus may spread from the germ end under the pericarp so as to discolor the lower half of the kernel.

Several samples of bin-selected ears showed cracking of kernels just above the cap, as illustrated in Plate 12, B. This cracking would involve as many as 8 or 10 adjacent kernels in a row. Such symptoms were found irregularly distributed throughout the ear. In the majority of such cases *Fusarium moniliforme* was found associated with such injury. The primary cause of this condition was not determined. The influence of excessive moisture and growth may have been the contributing factors. However, it would appear from all evidence that the fungus became established subsequent to the cracking. The embryo in these cracked kernels is frequently killed by the fungus. However, such seeds seldom develop into strong seedlings, owing to the amount of infection. A series of 20 cracked and 20 normal-appearing kernels from the same ear were planted in the greenhouse. Only 9 of the cracked kernels developed, whereas perfect germination and development were secured with the ones not cracked. During germination, when the plants are 6 to 8 inches high, it is found on removing the seedling that the seed coat is conspicuously discolored. The pericarp takes on a deep lavender color.

On the germinator, infection first appears as a slight effused growth around the cap. As a rule, the fungus ramifies between the pericarp and epidermis, resulting in pronounced discoloration of the pericarp and involving the lower half of the kernel. Often the discoloration will appear as irregular streaks, extending from the germ end toward the crown. The germination of corn is inhibited by this fungus but not as severely as was observed with *Gibberella saubinetii* and *Diplodia zeae*.

The kernel-rotted seeds (Pl. 12, E) show extensive ramification of the fungus. All parts of the kernels are invaded by the mycelium, which is intercellular as well as intracellular. The resultant effect is similar to the rot produced by *Gibberella saubinetii* on corn and wheat.

On culture media this fungus makes a slight to dense effused growth. With some strains there is a conspicuous pigmentation of the vegetative growth. The dense growth of mycelium often appears lavender in color, although this was not observed to be consistent for any one strain regardless of the medium. The catenulate conidia can easily be determined by examining the aerial growth with the low-power objective. The chains of spores vary in number, and the maximum counted was 30 spores in a single chain. It is not uncommon to find the conidia aggregated into heads, as shown in Plate 2, G-H. The conidiophores are simple or branched as shown in Plate 2, D-F. Strands of mycelium with numerous conidiophores often become interlaced. The conidia in chains are slightly pyriform, and the attenuated end of the spore is often sharply muticate. At time of germination they are considerably larger and often with one or two cross septa (Pl. 2, B). The following measurements of microconidia by various investigators are given for comparison:

Saccardo (*27*), host material	8 to 10μ by 2.5 to 3μ.
Sheldon (*29*)	6 to 10μ length.
Tiraboschi (*35*)	5 to 7μ by 2 to 3.5μ.
Hartley, Merrill, and Rhoads (*15*)	Prune agar 4.8 to 6.3μ by 2.2 to 3.1μ; corn meal 7.4 to 11.1μ length.
Authors, host material	7 to 9μ by 2.6 to 3.5μ.
Authors, dextrose medium	6.7 to 12μ by 2.5 to 3.5μ.

The macroconidia are sparingly developed and were first observed in cultures that were at least 2 months old. Some strains even as old as that failed to develop macroconidia. We did not observe any of the macroconidia on host material. Marcoconidia in our cultures were 3-septate and measured 19 to 31.5 μ by 2.5 to 3.5 μ. Cultures on rice agar were first found to produce macroconidia, and similar results were obtained with cultures on steamed corn meal.

DIPLODIA ZEAE (SCHW.) LEV.

The term "dry rot" is often used in describing the effects on the ears produced by this fungus. However, the term seems no more applicable in this case than for the kernel-rot produced by *Fusarium moniliforme* and *Gibberella saubinetii.*

In extreme infection of the ears the matting and rotting are similar to that described for *Gibberella saubinetii* (Pl. 13) and which by growers is called moldy ears. The mycelium is established in a like manner, but no pink coloration is produced, such as is often found with infection of *G. saubinetii.* Kernels of both white and yellow varieties of corn show considerable blackening, which varies from irregular streaks to large areas involving more or less the entire kernel. In some instances the development of pycnidia is so abundant as to produce this blackened appearance. It is not uncommon to find scattered infected kernels at the tip of ears of which the upper part is brown to black in color. The pericarp in this region is of a fragile texture, and inside is found a powdery brown mass consisting of corroded starch grains with abundant brown septate mycelium.

A study of diseased kernels in cross section has shown the fungus well established between the aleurone layer and integuments. Immature pycnidia have been observed in this region. The mycelium ramifies through the endosperm tissue. The starch grains appear corroded, as reported by Heald, Wilcox, and Pool (*16*). Rotted kernels present the same physical condition as that described for *Fusarium moniliforme*.

Kernels which appear normal but show infection during germination often produce black streaks at the germ end. This appearance results from developing and progressing of the fungus under the pericarp. An interlaced mass of brown mycelium is often found developing under the pericarp. The most conspicuous sign is found during germination with the effused growth of white, cottony mycelium at the germ end. Germination is greatly retarded and often inhibited as a result of infection. A summary of extensive germination tests shows that this fungus is more inhibitive to germination than the other parasites described.

The white, cottony effused growth of this fungus in agar cultures is easily determined. The mycelium on the substratum takes on a brownish appearance with age. On rice agar it fruits abundantly. The conidia (pycnospores) are 2-celled and brown in color. Measurements of conidia from infected roots and cultures are the same. Conidia from rice media measure 16.5 to 31.5 μ by 5 to 6 μ.

PREVALENCE

In making a survey of fungi internal of corn in Delaware an appeal was made to several hundred farmers, including many members of the State Corn Growers' Association, requesting at least four ears of seed corn, two of which should represent the best seed corn grown and two ears much above the average from bin selection; the object of making a request of this nature was to secure material from which first-hand information could be obtained on the amount of internal infection carried in seed corn and to learn whether field selection was superior to bin selection. In order to make a comparison with other States a request for cooperation was sent to pathologists and agronomists of 20 States of the corn belt.

A careful review of Table I indicates that there are at least four species of fungi commonly found internal of seed corn which are widely distributed. We fully realize that the survey if not extensive enough to show the condition prevailing in seed corn in the several districts throughout the United States. Superficially the survey shows that the organisms vary in prevalence considerably throughout the different sections. *Cephalosporium sacchari*, *Fusarium moniliforme*, and *Diplodia zeae* seem to have a wide range but are probably more prevalent through the middle tier of States and the southern States, whereas *Gibberella saubinetii* appears more prevalent through the States producing winter wheat.

In North Dakota, from which 25 samples were received out of which 375 kernels were cultured, no *Cephalosporium sacchari* or *Gibberella saubinetii* was found and only a trace of *Fusarium moniliforme* and *Diplodia zeae*. The samples from several or the States were not representative farm samples but were sent as special selections from experiment stations.

TABLE I.—*Survey by States of parasitic fungi internal of seed corn*

State.	Number of samples.	Number of kernels cultured.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Germination.		
							Strong.	Weak.	Dead.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Del	219	3,285	39.54	5.95	19.92	5.69	86.35	9.56	4.09
Ark	15	225	22.00	1.33	35.33	3.33	66.00	26.66	7.34
Conn	12	180	16.66	25.83	41.66	.....	84.16	14.16	1.68
Ill	25	375	2.00	.....	12.40	7.20	82.80	16.00	1.20
Ind	17	255	4.70	4.70	.....	.....	96.44	3.56	.....
Ky	34	510	3.82	4.11	17.94	4.85	59.70	15.59	24.71
Kans	12	180	11.66	.....	30.00	.....	88.33	11.67	.....
La	1	15	.....	.....	80.00	.....	100.00	.....	.....
Mass	5	75	.....	12.00	10.00	8.00	85.00	6.00	9.00
Md	10	150	46.00	7.00	12.00	14.00	65.00	35.00	.....
Minn	10	150	10.00	12.00	43.00	.....	37.00	55.00	8.00
Miss	16	240	38.13	.....	40.00	3.12	74.37	25.00	.63
Nebr	14	215	22.85	.....	20.00	1.42	85.71	4.28	10.01
N. C	10	150	38.00	2.00	48.00	.....	86.00	14.00	.....
N. Dak	25	375	.....	.....	.40	.80	85.60	1.20	13.20
N. J	10	150	24.00	21.00	17.00	2.00	79.00	21.00	.....
N. Y	6	90	.....	3.33	.....	.....	96.67	3.33	.....
Ohio	11	165	10.90	22.72	1.82	12.73	49.09	50.91	.....
Pa	14	210	5.71	7.87	.....	.....	89.57	3.57	6.86
Tex	7	105	14.28	.....	71.42	2.85	21.42	78.58	.....
Wis	7	105	20.00	2.85	.....	10.85	97.14	.....	2.86

Of the several parasitic fungi carrying internal of seed corn in Delaware *Cephalosporium sacchari* is most prevalent, occurring in the several thousand kernels cultured to the extent of 39.54 per cent. This fungus is frequently found associated in the same kernel with the other parasitic fungi *Fusarium moniliforme, Gibberella saubinetii, and Diplodia zeae.*

Fusarium moniliforme as an internal parasite is found to the extent of 19.92 per cent in seed corn in Delaware. Its latitude or range of infection is the same as that of *Cephalosporium sacchari*, and frequently both are found occupying the same kernels. The fact that *C. sacchari* and *F. moniliforme* fruit abundantly both internally and externally of seed corn would indicate that surface contamination must be very common.

Gibberella saubinetii is much less common as an internal parasite of corn in Delaware and throughout the United States than either *Cephalosporium sacchari* or *Fusarium moniliforme.* In Delaware 5.95 per cent of the kernels cultured gave this fungus. The fact that *G. saubinetii* fruits more sparingly as an ear rot and kernel infection of corn and produces no chlamydospores would indicate that as a seed-carrying organism its possibility of distribution is much more limited than that of either *C. sacchari* or *Fusarium moniliforme.* Its prevalence seems to vary greatly in the several districts.

Diplodia zeae like the other parasites considered above may be internal of seed corn that appears entirely healthy. Its prevalence in Delaware seed corn for 1920 was 5.69 per cent. As a parasite its range of activity, judging from internal infection, is identical with that of *Cephalosporium sacchari* and *Fusarium moniliforme*—probably not quite so great in the Gulf States as in the Central States.

Species of the following fungi in several instances also have been found internal of seed corn: Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus, Torula, Hormodendron, Chaetomium, Helminthosporium, and Spicaria. *Colletotrichum cereale* has also been found. Adams and Russell (*2*) have shown *Rhizopus nigricans* to be a parasite of the storage tissue of the scutellum and a limiting factor in germination. The junior writer has also noted the same condition for species of Penicillium and Aspergillus. It is possible the other forms are likewise concerned with inhibiting germination. Unlike the four species described above, these fungi are not so consistently found associated with seed corn, hence are of much less importance. Also several times we have met with two species of bacteria, a yellow organism and a white organism; the importance of these we have not determined. The extent of these associated fungi and bacteria as internal organisms is given in detail in the Table II, which shows the general distribution and prevalence of fungi internal of seed corn by samples from the several States.

By a careful study of Table II it is occasionally seen that there are samples of corn running low in germination that contain no internal parasites. The explanation of this may be root rot, so weakening the plants as to effect germination, or possibly storage difficulties which reduced viability. As a rule, weakened or impaired germination seems to be associated with internal infection, the most inhibiting parasites being *Diplodia zeae* and *Gibberella saubinetii*.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	DELAWARE							
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1A	Yellow Dent, local	60	.....	20	.....	.....	90	10
1B	.....do	.....	.....	.....	.....	.....	100	.....
1C	.....do	.....	.....	.....	.....	.....	100	.....
1D	.....do	40	.....	.....	.....	.....	100	.....
2A	Boone County White	100	.....	.....	.....	.....	90	10
2B	.....do	20	.....	.....	.....	.....	80	10
2C	.....do	.....	.....	.....	.....	.....	100	.....
2D	.....do	.....	20	.....	.....	.....	90	.....
3A	Johnson County White	20	.....	25	.....	[10] 30	100	.....
3B	.....do	100	.....	.....	20	.....	100	.....
3C	.....do	100	.....	.....	.....	.....	100	.....
3D	.....do	100	.....	.....	.....	.....	100	.....
4A	Yellow Dent, local	100	.....	.....	.....	.....	80	.....
4B	.....do	80	.....	20	.....	.....	100	.....
4C	.....do	100	.....	50	.....	.....	100	.....
4D	.....do	100	.....	80	.....	.....	100	.....
5A	White Dent, local	.....	.....	.....	.....	.....	100	.....
5B	.....do	60	.....	.....	.....	.....	100	.....
6A	.....do	.....	.....	.....	.....	.....	100	.....
6B	.....do	100	.....	.....	.....	.....	100	.....
7A	Reid Yellow Dent	.....	.....	.....	60	.....	100	.....
7B	.....do	.....	.....	.....	80	.....	70	30

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*. 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubientii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	DELAWARE—continued	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
8A	Reid Yellow Dent	.....	.....	.....	60	[10] 40	100	.....
8B	.....do	100	.....	.....	40	.....	70	30
9A	Yellow Dent, local	20	.....	20	20	.....	100	.....
9B	.....do	60	.....	60	.....	.....	100	.....
10A	.....do	100	40	.....	.....	.....	100	.....
10B	.....do	80	20	.....	70	.....	30	.....
11A	Early Bristol	.....	.....	.....	20	.....	100	.....
11B	.....do	80	.....	40	.....	.....	100	.....
12A	.....do	100	.....	40	.....	.....	100	.....
12B	.....do	100	.....	40	.....	.....	100	.....
13A	White Dent, local	60	.....	.....	.....	.....	100	.....
13B	.....do	100	.....	20	.....	.....	100	.....
14A	.....do	.....	.....	.....	.....	.....	90	.....
14B	.....do	100	.....	.....	.....	.....	100	.....
15A	Yellow Dent, local	80	.....	.....	.....	.....	100	.....
15B	.....do	.....	.....	.....	.....	.....	100	.....
15C	Yellow Dent, local	80	.....	.....	.....	.....	100	.....
15D	.....do	20	.....	.....	.....	[10] 20	100	.....
16A	White Capped Yellow Dent, local.	20	.....	.....	.....	.....	100	.....
16B	.....do	100	40	.....	.....	.....	100	.....
17A	.....do	60	.....	40	.....	.....	100	.....
17B	.....do	100	.....	.....	10	.....	70	30
18A	.....do	80	.....	20	.....	.....	100	.....
18B	.....do	.....	.....	.....	.....	.....	100	.....
18C	.....do	80	.....	20	.....	.....	100	.....
18D	.....do	40	.....	20	20	.....	100	.....
19A	White Dent, local	.....	.....	20	.....	.....	100	.....
19B	.....do	60	20	.....	.....	.....	100	.....
19C	.....do	100	60	60	.....	.....	80	.....
19D	.....do	40	.....	.....	.....	.....	100	.....
20	Yellow Dent, local	.....	.....	100	.....	.....	80	.....
21A	.....do	100	.....	.....	.....	.....	100	.....
21B	.....do	.....	.....	80	.....	.....	100	.....
21C	.....do	100	.....	.....	.....	.....	70	30
21D	.....do	60	.....	60	.....	.....	100	.....
21E	.....do	100	.....	.....	.....	.....	70	30
22A	.....do	.....	.....	.....	.....	.....	100	.....
22B	.....do	100	.....	.....	.....	[7] 60	100	.....
22C	.....do	.....	20	.....	.....	[3] 50	72	.....
23A	White capped Yellow Dent, local	.....	.....	.....	.....	.....	100	.....
23B	.....do	.....	.....	.....	.....	.....	90	10
24A	.....do	.....	40	.....	20	.....	10	70
24B	.....do	60	.....	.....	.....	.....	100	.....
25A	Johnson County White	60	.....	.....	.....	.....	10	90
25B	.....do	20	.....	.....	.....	.....	90	.....
25C	.....do	.....	.....	.....	.....	.....	100	.....
25D	.....do	20	.....	.....	.....	.....	100	.....
26A	Reid Yellow Dent	20	.....	.....	.....	.....	100	.....
26B	.....do	20	.....	.....	.....	.....	90	.....

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sec-chari.*	*Gibberella saubientii.*	*Fusarium moniliforme.*	*Diplodia forme.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	DELAWARE—continued	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
27A	Reid Yellow Dent	.....	.....	.....	.....	[3] 20	100	.....
27B	.....do	.....	.....	.....	.....	[7] 60	60	30
28A	Yellow Dent, local	100	.....	30	.....	.....	100	.....
28B	.....do	100	.....	20	.....	.....	100	.....
28C	.....do	80	.....	20	.....	.....	100	.....
28D	.....do	20	.....	100	40	.....	90	.....
29A	Reid Yellow Dent	30	.....	100	.....	.....	100	.....
29B	.....do	100	.....	.....	.....	.....	100	.....
30A	.....do	.....	100	.....	.....	.....	100	.....
30B	.....do	100	100	.....	.....	.....	100	.....
31A	White Dent, local	.....	.....	80	.....	.....	50	.....
31B	.....do	.....	25	.....	.....	.....	70	30
31C	.....do	100	.....	40	.....	.....	100	.....
31D	.....do	60	20	10	.....	.....	60	30
32A	.....do	100	40	100	.....	.....	90	.....
32B	.....do	30	.....	30	20	.....	100	.....
32C	.....do	100	40	10	.....	.....	90	.....
32D	.....do	100	100	20	.....	.....	100	.....
33A	.....do	60	.....	40	60	.....	.....	40
33B	.....do	80	70	20	.....	.....	100	.....
33C	.....do	.....	.....	.....	.....	.....	100	.....
33D	.....do	60	.....	.....	80	.....	.....	40
33E	.....do	100	.....	60	.....	.....	100	.....
34A	Reid Yellow Dent	60	.....	20	.....	.....	80	.....
34B	.....do	.....	.....	80	.....	.....	80	.....
34C	.....do	.....	.....	80	.....	.....	80	20
34D	.....do	.....	.....	100	.....	.....	40	40
34E	.....do	60	.....	100	.....	.....	60	30
35A	White Capped Yellow Dent, local	.....	.....	20	10	.....	50	30
35B	.....do	20	.....	.....	.....	.....	100	.....
35C	.....do	.....	40	.....	.....	.....	60	40
35D	.....do	.....	40	.....	.....	.....	100	.....
36A	.....do	.....	10	20	.....	.....	30	50
36B	.....do	.....	.....	.....	.....	.....	10	90
37A	.....do	.....	.....	.....	.....	.....	100	.....
37B	.....do	.....	.....	.....	.....	.....	100	.....
38A	.....do	.....	.....	.....	.....	.....	80	20
38B	.....do	50	.....	88	20	.....	100	.....
38C	.....do	.....	.....	60	.....	.....	100	.....
39A	.....do	60	.....	.....	.....	.....	70	30
39B	.....do	40	.....	.....	.....	.....	100	.....
40A	White Dent, local	20	.....	.....	.....	.....	100	.....
40B	.....do	40	.....	.....	.....	.....	100	.....
40C	.....do	.....	.....	.....	.....	.....	100	.....
40D	.....do	20	.....	.....	.....	.....	100	.....
40E	.....do	60	.....	40	.....	.....	100	.....
41A	.....do	20	.....	10	10	.....	90	.....
41B	.....do	.....	40	.....	10	.....	70	.....
41C	.....do	20	40	.....	.....	.....	90	.....
42A	Yellow Dent, local	40	.....	40	60	.....	50	.....
42B	.....do	100	.....	.....	.....	.....	70	30

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Peinicillum* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.	Germination.	
							Strong.	Weak.
	DELAWARE—continued							
43A	White Capped Yellow Dent, local	Per ct.	Per ct.	Per ct.	Per ct. 60	Per ct.	Per ct. 100	Per ct.
43B	do						40	60
43C	do						100	
43D	do	20					100	
44A	Yellow Dent, local	80		40			90	
44B	do	40	20				100	
45A	do	100		20			100	
45B	do		50	40			100	
46A	Johnson County White						100	
46B	do						100	
46C	do						100	
46D	do						100	
46E	do						100	
46F	do						100	
47A	Cloud's Yellow Dent		20				90	
47B	do						100	
47C	do						100	
47D	do						100	
48A	Yellow Dent, local						100	
48B	do						40	40
48C	do			40			90	10
48D	do	20		40			100	
49A	White Dent, local	60		60			100	
49B	do	60		60			90	
49C	do	100		100			100	
49D	do	100					100	
50	Illinois Low Oil	60		20			70	
51	White Dent, local	100		75	25		100	
52	do	100		75	40		40	30
53	do	25		100			100	
54	do	60		60	40		20	80
55	do				40		40	
56	do	30		66			100	
57	do	50			10		100	
58	do	100		50	40		40	60
59	do	40		40			90	10
60	do	60		40			100	
61	do	100					100	
62	do	100		20			100	
63	do	80		80			100	
64	do	100					100	
65	do	100		100			60	40
66	do	100					100	
67	do			100			30	70
68	do				100		100	
69	do			25			100	
70	do						100	
71	do	20		50	40		80	20
72	do	100					70	30
73	do			100			100	

[a] Letters A, B, C, D, etc., indicate different ears from same source.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	DELAWARE—continued	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
74	White Dent, local	.....	.....	.....	.....	[1]10	100	.....
75A	.....do	40	.....	.....	.....	.....	100	.....
75B	.....do	10	.....	20	.....	.....	100	.....
75C	.....do	40	.....	40	.....	.....	100	.....
75D	.....do	60	.....	10	.....	[3]40	80	10
76A	Yellow Dent, local	.....	40	20	.....	.....	90	10
76B	.....do	.....	.....	20	.....	[3]100	100	.....
76C	.....do	.....	.....	.....	.....	.....	100	.....
77	White Dent, local	.....	.....	.....	.....	.....	100	.....
78A	.....do	20	.....	.....	20	.....	100	.....
78B	.....do	20	.....	.....	.....	.....	100	.....
78C	.....do	40	.....	.....	.....	.....	100	.....
78D	.....do	100	.....	.....	.....	[12]100	100	.....
79A	Yellow Dent. local	20	.....	100	.....	.....	10	90
79B	.....do	20	.....	.....	.....	[9]40	40	60
79C	.....do	100	.....	.....	.....	.....	60	40
79D	.....do	.....	.....	.....	.....	.....	100	.....
80A	Golden Beauty	.....	80	.....	.....	.....	70	30
80B	.....do	.....	.....	.....	.....	.....	.....	100
80C	.....do	.....	.....	.....	20	.....	60	40
80D	.....do	.....	.....	.....	.....	[3]20	100	.....
80E	.....do	.....	.....	.....	.....	{[11]20 [12]20}	100	.....
80F	.....do	.....	.....	.....	.....	.....	100	.....
80G	.... do	.....	.....	.....	.....	.....	100	.....
80H	.....do	.....	.....	.....	.....	.....	60	40
81A	White Dent, local	.....	.....	.....	20	.....	40	60
81B	.....do	.....	.....	20	.....	.....	70	30
81C	....do	.....	.....	100	.....	.....	20	80
81D	.....do	.....	80	80	.....	.....	30	70
81E	.....do	20	.....	100	.....	.....	50	50
82A	Bristol Dent	40	.....	.....	.....	[3]60	100	.....
82B	.....do	100	.....	.....	.....	.....	100	.....
82C	.....do	40	.....	.....	.....	.....	100	.....
		100	.....	.....	.....	.....	100	.....
	ARKANSAS							
83	Washington County	20	.....	60	.....	.....	70	30
84	Paymaster	40	.....	10	.....	.....	80	20
85	Whartley's Prolific	30	.....	20	.....	.....	90	.....
86	Hackberry	20	20	100	.....	.....	10	90
87	McFarland	.....	.....	20	.....	.....	30	70
88	Southern Beauty	.....	.....	40	.....	.....	60	40
89	Chesholm	60	.....	40	10	.....	30	70
90	U. S. 165	.....	.....	.....	10	.....	50	50
91	Stewarts Yellow Dent	20	.....	60	.....	.....	100	.....
92	U. S. Sel. No. 77	.....	.....	20	.....	[1]20	.....	.....
93	Experiment Station Yellow	.....	.....	40	.....	.....	100	.....
94	Boone County White	100	.....	80	.....	.....	90	10
95	Laguna Mexican June	20	.....	40	10	.....	90	10
96	Biggs–Seven–Ear	.....	.....	.....	20	.....	100	.....
97	Eureka	20	.....	.....	.....	.....	90	10

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubientii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	CONNECTICUT	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
98	Bearsley's Leaming						100	
99	Webber's Dent		50				30	70
100	Early Lakeside		10			[4] 30		80
101	King Philip		10				100	
102	Sharon White Cap						100	
103	Pride of North		70				100	
104	Golden Nugget	20	10				100	
105	Sanford White		50				100	
106	Longfellow		60					100
107	Rhode Island White		20	40			100	
108	Century Dent			10			100	
109	Twelve Row		30				100	
	ILLINOIS							
110	Western Plowman						70	30
111	Reid Yellow Dent						100	
112	.....do			40	20	[3] 20	100	
113	.....do				20		70	30
114	.....do				20		60	40
115	.....do					[4] 10	60	40
116	Leaming			20			30	70
117	Boone County White						100	
118	Silvermine						100	
119	Champion White Pearl			20			20	80
120	Illinois High Oil						100	
121	Illinois Low Oil						100	
122	Illinois High Protein			20	20		100	
123	Illinois Low Protein				40		60	40
124	Yellow Dent			40	40		60	40
125	White Dent	30		10			100	
126	Variety not given			40			100	
127	.....do			60		[12] 20	90	10
128	.....do						100	
129	.....do						100	
130	Yellow Dent				20		80	
131	.....do					[10] 20	100	
132	.....do			60			80	20
133	Variety not given	20					90	
134	.....do						100	
135	Colclessons White Dent			20			100	
	INDIANA							
136	Johnson County Yellow Dent						100	
137	Johnson County White Dent					[11] 20	100	
138	Reid Yellow Dent						100	
139	Vogliss White Dent			20		[4] 20	100	
140	Reid Yellow Dent						100	
141	Johnson County White Dent						100	
142	.....do						100	
143	Colclessons White Dent	80					80	20

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[b] The small numbers in this column refer to the following numbered fungi: 1. *Aspergillus* spp.; 2. *Cladosporium* spp.; 3. *Penicillium* spp.; 4. *Alternaria* spp.; 5. *Helminthosporium* spp.; 6. *Rhizopus* spp.; 7. *Sphaeria* spp.; 8. *Hormodendron* spp.; 9. *Torula* spp.; 10. *Chaetomium* spp.; 11. *Colletotrichum cereale*; 12. *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	INDIANA—continued							
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
144	Reid Yellow Dent		20				100	
145	Johnson County White Dent		60				60	40
146	Voglers White Dent						100	
147	Yellow						100	
148	Flesh Corn						100	
149	Yellow						100	
150	White						100	
151	Yellow						100	
	KANSAS							
152	Pride of Salem						100	
153	.....do						100	
154	.....do			60			100	
155	.....do			60			100	
156	.....do	40		40			70	30
157	.....do			20			90	10
158	.....do			60			70	30
159	.....do	20		20			90	10
160	.....do	80		40			80	20
161	.....do						100	
162	.....do			60			60	40
163	.....do						100	
	KENTUCKY							
164	Variety not given						100	
165	.....do						100	
166	.....do		100				60	40
167	.....do			20	10		60	40
168	.....do			40			100	
169	.....do			40			60	40
170	.....do			10			90	10
171	.....do			20			80	20
172	.....do	20		10	10		40	60
173	.....do			10	20		30	70
174	.....do			40	40		40	60
175	.....do			20	40		100	
176	.....do						100	
177	.....do	20		20			90	
178	.....do			10	10		90	
179	.....do		10	40		[3] 10	80	
180	.....do			100			40	60
181	.....do	20					100	
182	.....do	40					100	
183	.....do						90	10
184	.....do			10			100	
185	.....do	20					100	
186	.....do			30			10	90
187	.....do	20		20	20		70	30
188	.....do				10		100	
189	.....do						100	

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1 *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	KENTUCKY—continued	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
190	Variety not given	.....	.....	60	.....	[1] 100	.....	.....
191	.....do	.....	.....	.....	.....	[1] 1, 30	.....	.....
192	.....do	.....	.....	.....	.....	[1] 1, 20	.....	.....
193	.....do	.....	.....	70	.....	[1] 100	.....	.....
194	.....do	10	10	40	.....	[1] 100	.....	.....
195	.....do	.....	.....	10	.....	[1] 100	.....	.....
196	.....do	.....	.....	.....	5	.....	.....	.....
197	.....do	.....	.....	.....	.....	[1] 10	.....	.....
	LOUISIANA							
198	Calhound White	.....	.....	80	.....	.....	100	.....
	MARYLAND							
199	Yellow Dent, local	100	.....	.....	.....	.....	80	30
200	.....do	40	.....	.....	.....	.....	100	.....
201	.....do	.....	.....	.....	.....	.....	100	.....
202	.....do	.....	.....	.....	.....	.....	100	.....
203	.....do	20	.....	.....	.....	.....	100	.....
204	.....do	60	.....	40	60	.....	50	50
205	.....do	80	70	20	.....	.....	100	.....
206	.....do	.....	.....	.....	.....	.....	20	80
207	.....do	60	.....	.....	80	.....	50	50
208	.....do	100	.....	60	.....	.....	50	50
	MASSACHUSETTS							
209	Sweet Corn	.....	20	.....	20	[4] 30	70	
210	Yellow	.....	.....	20	.....	.....	80	20
211	Boone County White	.....	.....	20	20	.....	90	10
212	White Dent	.....	.....	.....	.....	.....	100	.....
213	Yellow Dent	.....	40	10	.....	[4] 10	80	.....
	MINNESOTA							
214	Variety not given	.....	.....	100	.....	[3] 60	.....	100
215	.....do	60	.....	50	.....	.....	100	.....
216	.....do	.....	.....	.....	.....	[3] 100	.....	20
217	.....do	.....	80	20	.....	.....	100	.....
218	.....do	.....	.....	50	.....	.....	50	50
219	.....do	.....	40	30	.....	[11] 60	20	80
220	.....do	.....	.....	100	.....	.....	20	80
221	.....do	.....	.....	20	.....	[3] 100	40	60
222	.....do	40	.....	20	.....	.....	20	80
223	.....do	.....	.....	40	.....	[11] 40	20	80
	MISSISSIPPI							
224	Paymaster (Harpeth)	10	.....	40	.....	.....	60	40
225	Williamson (Cokers)	60	.....	60	.....	.....	100	.....
226	Ellis (Cokers)	40	.....	60	.....	.....	60	40

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TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	MISSISSIPPI—continued							
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
227	Ewing's Mosbys (Station)	60	.....	40	.....	.....	100	.....
228	Mosbys (Cokers)	40	.....	60	.....	.....	70	30
229	Hastings	.....	.....	10	10	.....	80	10
230	Station Laguna	60	.....	80	.....	.....	50	50
231	Chesholm (Ferg.)	40	.....	40	.....	.....	100	
232	Marlboro (Wann)	40	.....	40	.....	.....	90	10
233	Paymaster (Neals)	10	.....	70	10	.....	30	70
234	North Carolina Prolific	10	.....	60	10	.....	40	60
235	Station Tennessee Red Cob	20	.....	.....	.....	.....	80	20
236	Station Tennessee Red Cob 72	100	.....	40	.....	.....	60	40
237	Station Florida Flint	100	.....	.....	.....	.....	90	10
238	Whatley's Prolific	20	.....	40	20	.....	90	10
239	Reid Yellow Dent	.....	.....	.....	.....	.....	90	10
	NEBRASKA							
240	Yellow Dent, local	80	.....	20	.....	.....	80	20
241	.....do	.....	.....	.....	.....	.....	100	.....
242	.....do	.....	.....	40	.....	.....	90	.....
243	.....do	.....	.....	40	.....	.....	70	30
244	.....do	40	.....	20	.....	.....	100	.....
245	.....do	40	.....	40	.....	.....	100	.....
246	White Dent, local	.....	.....	.....	.....	.....	.....	.....
247	Haynes Yellow Dent	20	.....	20	.....	.....	90	.....
248	Yellow Dent, local	.....	.....	.....	.....	.....	100	.....
249	.....do	40	.....	20	.....	.....	80	.....
250	.....do	.....	.....	.....	.....	[11] 20	100	.....
251	Calico Dent, local	80	.....	60	.....	.....	100	.....
252	White Cap Dent, local	.....	.....	.....	.....	[10] 20	100	.....
253	Nebraska White Prize	20	.....	20	20	.....	90	10
	NEW JERSEY							
254	Variety not given	.....	.....	.....	.....	.....	50	50
255	.....do	.....	60	.....	.....	.....	90	10
256	.....do	.....	10	10	.....	.....	100	.....
257	.....do	.....	60	20	.....	.....	20	80
258	.....do	20	20	.....	.....	.....	80	20
259	.....do	100	20	20	.....	[4] 20	80	20
260	.....do	.....	.....	.....	.....	.....	100	.....
261	.....do	100	20	20	.....	.....	90	10
262	.....do	.....	20	100	20	[4] 20	80	20
263	.....do	20	.....	.....	.....	.....	100	.....
	NEW YORK							
264	Variety not given	.....	.....	.....	.....	.....	100	.....
265	.....do	.....	.....	.....	.....	.....	100	.....
266	.....do	.....	.....	.....	.....	.....	100	.....
267	.....do	.....	.....	.....	.....	.....	100	.....
268	.....do	.....	20	.....	.....	.....	80	20
269	.....do	.....	.....	.....	.....	.....	100	.....

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[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	NORTH CAROLINA	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
270	Neals Paymaster	.....	.....	80	.....	.....	20	80
271	Deatons Two Ear	20	.....	60	.....	.....	100	.....
272	Cooke's Prolific	.....	.....	40	.....	.....	100	.....
273	Long Valley White	60	.....	40	.....	.....	40	60
274	Grampian	.....	.....	.....	.....	.....	100	.....
275	Weekleys Improved	60	.....	100	.....	.....	100	.....
276	Biggs Seven Ear	80	.....	20	.....	.....	100	.....
277	Horse-Tooth	60	20	40	.....	.....	100	.....
278	Kickers Indian-Chief	.....	.....	60	.....	.....	100	.....
279	Lathanis Double	100	.....	40	.....	.....	100	.....
	NORTH DAKOTA							
280	Variety not given	.....	.....	.....	.....	.....	100	.....
281	.....do	.....	.....	10	.....	.....	100	.....
282	.....do	.....	.....	.....	.....	.....	100	.....
283	.....do	.....	.....	.....	.....	.....	100	.....
284	.....do	.....	.....	.....	.....	.....	100	.....
285	.....do	.....	.....	.....	.....	.....	100	.....
286	.....do	.....	.....	.....	20	.....	80	.....
287	.....do	.....	.....	.....	.....	.....	100	.....
288	Minnesota No. 13	.....	.....	.....	.....	.....	100	.....
289	.....do	.....	.....	.....	.....	.....	100	.....
290	.....do	.....	.....	.....	.....	.....	100	.....
291	.....do	.....	.....	.....	.....	.....	.....	(c)
292	A Yellow Capped Red	.....	.....	.....	.....	.....	.....	(c)
293	Northwestern Dent	.....	.....	.....	.....	[8] 100	[11] 100	(c)
294	.....do	.....	.....	.....	.....	.....	100	.....
295	.....do	.....	.....	.....	.....	[12] 20	100	.....
296	White Rustler	.....	.....	.....	.....	.....	100	.....
297	.....do	.....	.....	.....	.....	.....	100	.....
298	.....do	.....	.....	.....	.....	.....	100	.....
299	Yellow Dent	.....	.....	.....	.....	.....	100	.....
300	.....do	.....	.....	.....	.....	.....	100	.....
301	Labels lost in shipment	.....	.....	.....	.....	.....	100	.....
302	.....do	.....	.....	.....	.....	.....	70	30
303	.....do	.....	.....	.....	.....	.....	100	.....
304	.....do	.....	.....	.....	10	.....	90	.....
	OHIO							
305	White Capped Red	.....	70	.....	.....	.....	70	30
306	.....do	20	.....	.....	100	.....	.....	100
307	.....do	.....	20	.....	.....	.....	.....	100
308	Bloody Butcher	.....	40	.....	.....	.....	100	.....
309	Yellow Dent, local	.....	.....	.....	40	.....	70	30
310	White Cap	.....	.....	.....	.....	.....	100	.....
311	Snures White Cap	.....	100	.....	.....	.....	30	70
312	Stauffers Yellow Cap	.....	20	.....	.....	.....	100	.....
313	J. S. Leaming	100	.....	20	.....	.....	30	70
314	Clarage	.....	.....	.....	.....	[3] 20 [12] 20	20	80
315	Stauffers Yellow	.....	.....	.....	.....	.....	20	80

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[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Spicaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

[c] Probably all frosted; dead.

TABLE II.—*Distribution and prevalence of fungi internal of seed corn*—Continued

No.[a]	State and variety.	*Cephalosporium sacchari.*	*Gibberella saubinetii.*	*Fusarium moniliforme.*	*Diplodia zeae.*	Other fungi.[b]	Germination.	
							Strong.	Weak.
	PENNSYLVANIA							
316	Johnson County White	40					100	
317	Landis White		10			1 10 3 20	90	10
318	Reid Yellow Dent	20					90	10
319	College White Cap						100	
320	Greene County Yellow Dent	20					100	
321	Clouds Yellow Dent		100					20
322	Golden Yellow Dent						100	
323	.....do						100	
324	.....do						90	10
325	.....do					3 90	80	
326	Lags Clapper						100	
327	Johnson County White						100	
328	.....do						100	
329	.....do						100	
	TEXAS							
330	Johnson County White	60		50		1 20 3 20	20	80
331	Chesholm			40	20	1, 3 60	20	80
332	Sure Cropper			100		1 80		6 100
333	.....do	20		60		3 60	60	40
334	.....do	20		70		1, 3 40		100
335	.....do			80		1, 3 75	20	80
336	.....do			100		1 40	30	70
	WISCONSIN							
337	Variety not given						100	
338	.....do	100					100	
339	.....do						100	
340	.....do						100	
341	.....do						100	
342	.....do						100	
343	.....do	40	20		60		80	

[a] Letters A, B, C, D, etc., indicate different ears from same source.

[b] The small numbers in this column refer to the following numbered fungi: 1, *Aspergillus* spp.; 2, *Cladosporium* spp.; 3, *Penicillium* spp.; 4, *Alternaria* spp.; 5, *Helminthosporium* spp.; 6, *Rhizopus* spp.; 7, *Sticaria* spp.; 8, *Hormodendron* spp.; 9, *Torula* spp.; 10, *Chaetomium* spp.; 11, *Colletotrichum cereale*; 12, *Bacterium* spp.

INTERNAL INFECTION

The evidence of internal infection in seed corn showing no external symptoms is suggestive with germinating kernels where the growth of the parasitic fungi is consistently observed at the germinal end. Even this evidence allows for error because of the possibility of surface organisms being present. In resorting to methods of thorough disinfection one still finds the presence of fungous growth appearing on the germinating seeds. The manner in which infection is carried in the seed has not heretofore been extensively investigated. Symptoms of kernels, as we have pointed out for the four parasites described, are easily recognized; however, it is with normal-appearing kernels that we wish to establish the manner of internal infection.

A brief discussion of the morphology of corn may help in explaining the exact nature of this internal parasitism. The kernel, seed, or corn to which it is commonly referred is a ripened fruit or caryopsis. It consists of a mature ovary with all its adnate parts. The kernel is established in a "fruit cup" formed by six fruit envelopes. According to Winton (*39*) this cup consists of—

> three glumes—a flowering glume, a palet, and another palet belonging originally to a rudimentary blossom.

The cap of the kernel consists of a dried tissue, nonfunctioning at maturity. The peripheral cells of the cap are continuous with those of the pericarp. They are approximate to the integuments on line with the base of the scutellum. The integuments curve in towards the dorsal end of the scutellum. The inner structure of the cap consists of thick-walled cells and several vascular strands. This cap if partly cut or broken off exposes a small cavity over the dorsal extremity of the scutellum. There is also observed a brown covering adhering to the lower dorsal end of the scutellum. The cavity varies in size, and the covering over the scutellum is light brown to black, according to variety.

The vascular bundles in the cob above the butt have a peripheral distribution, so that the center or pith is made up of only parenchyma cells. The attachment of a pair of ovaries converge to a common base in relation to the vascular system. The ovaries are in direct relation to several bundles which gradually converge into several strands near the base of each ovary. This relation in a young cob can be seen with a hand lens. It is mentioned briefly here since it explains the path of infection if it occurs through the vascular system. If such infection resulted it would seem that the pairs of kernels would be equally infected.

The four fungi mentioned have been found commonly established within kernels which show no symptoms of disease. It was further impossible in many cases to correlate any of the various symptoms of the butts of ears with such infection. The mycelium is found extensively developed in this cavity. In *Fusarium moniliforme* and *Cephalosporium sacchari* abundant internal spore production is found (Pl, 7, A). This can easily be determined with a hand lens or with the low-power objective of the microscope. In some instances where the fungus has progressed to the tissue of the embryo in kernels showing no external symptoms, a free-hand longitudinal section will show a discoloration of the embryo as well as fungus development in the cavity around the radicle and the plumule (Pl. 8 A, B). The development of the fungus from the cap into the germ and endosperm appears to be restricted by the black layer. However, with favorable conditions upon the germinator the fungus becomes active and the seedling is attacked. The feeders, scutellum, and epicotyl first show symptoms of infection.

This internal method of infection was first observed by the junior writer in the spring of 1920. It was observed that when the cap was removed increased germination free from severe infection was obtained. This point was thought to be the result of eliminminating the greater part of the internal fungus infection. It was further established that by disinfecting kernels after removal of cap perfect germination was secured in many instances. Where infection of *Fusarium moniliforme* and *Cephalosporium sacchari* occur the internal development in the cavity under the cap can be recognized with a hand lens. In *F. moniliforme* and *C. sacchari* the abundant internal development of spores is easily determined by the method of crushing the lower germinal end of the kernel and making

a poured agar plate. The numerous colonies appearing indicate the abundant internal spore production.

Many samples of seed corn show slight discoloration of the seed coat near the germinal end. These discolorations become pronounced during germination and assume various colors such as pink, lavendar, or black. The black discolorations were found most consistently with Diplodia infection, the lavendar with *Fusarium moniliforme*, and the pink with *Gibberella saubinetii*. This condition we consider the result of the fungus progressing beyond the cavity and becoming established in the tissue comprising the pericarp. We have observed this condition in kernels before and during germination. This method of internal infection explains the failure to secure disease-free seed by methods of disinfection. The tissue of the cap, even with extensive presoaking, fails to soften materially. The long exposures to various disinfectants are not entirely effective even subsequent to presoaking. Such treatments usually injure the embryo and retard germination.

Cross and longitudinal sections through the germ end of those internally infected kernels show the mycelium also established in the tissue of the cap. In some cases the mycelium is observed among the thin-walled cells near the periphery of the cap. The mycelium is not abundantly established, but it is observed ramifying between as well as penetrating the cells. The thick-walled cells toward the vascular strands do not appear to be penetrated. In several instances long intercellular hyphae were observed in the vascular tissues. The development of the fungus in the cavity is probably subsequent to the infection of the tissue comprising the cap. So far in our studies there was no conclusive evidence to show that such internal infection was always by means of the vascular system as the result of stalk or shank infection.

GENERAL DISCUSSION

With the nature of the internal infection determined, the means by which the fungus gains entrance and its relation to methods of control remain to be established.

According to the occurrence of seed corn infection, we must reckon with two means of infection quite independent of each other.

First: Direct kernel or blossom infection. Silk as the path of infection has been considered a common method for *Diplodia zeae* by Heald, Wilcox, and Pool (*16*) and Van Der Bijl (*38*) and for *Fusarium moniliforme* by Valleau (*36*). No conclusive proof has been advanced to show that infection occurs by means of the stigma. It is considered, in view of the work so far reported, that such infection occurs because the silk stage offers an accessible means of entrance. The silk, becoming dry and shriveling when the corn is in the late milk or dough stage, affords an opportunity for infection by wind-borne spores. Such type of infection apparently accounts for the ears shown in Plate 13. A parallel type of infection appears to be the most common for wheat scab as reported by Adams (*1*). Moisture conditions in the cup before maturity of the ear are no doubt favorable for development of the fungi once they are established from external sources. This tissue of the cap offers little resistance to these parasites and would allow for their establishment and development into the cavity under the cap. The prevalence of infection under these conditions would depend upon exposures of ears in the field to favorable conditions for infection. The study of infected kernels failed to indicate whether infection resulted by this means or from vas-

cular infection through the butt. Even after maturity if the corn is exposed to excessive or constant moisture, the various parasites as well as saprophytes may be established in the storage tissue of the fruit. This we believe in part explains the presence of *Aspergillusniger* and *Penicillium* spp. that frequently appear even after thorough disinfection. They have become established because of unfavorable conditions under which the corn has been exposed and stored. These types of fungi on the germinator may also retard or in some cases inhibit germination. They attack the storage tissue of the scutellum in a way similar to *Rhizopus nigricans*, as described by Adams and Russell (*2*). It is noted that where extensive rotting of kernels occurs on ears it is usually confined to the upper end. The exception in the case of the *F. moniliforme* kernel-rot is difficult to explain. The irregular rotted kernels, as shown in Plate 12, E, may have been individual grains delayed in ripening or ones for some unknown reason susceptible to infection. It is still an open question as to the possibility of direct silk infection.

Second: Indirect seed infection by means of systemic, stalk or shank infection. Smith and Hedges (*31*) have advanced some evidence of systemic infection for *Diplodia zeae*. If such type of infection commonly occurs with these parasites it would seem reasonable to expect poorer stands of corn than are reported. There is no doubt that a systemic infection occurs, but how commonly remains to be determined. Such infection does not necessarily have to follow from the planting of infected seed but may also occur because of soil infestation. Regardless of root infection, the stalk or shank may become points of initial infection. The fungus may in this way follow the vascular tissue into the fruit. This may be the most common means of infection and may explain the internal infection in seeds showing no external symptoms. Hoffer and Holbert (*18*) state that—

> after the first killing frost the stalks and especially the shanks and the ears are invaded rapidly by these disease organisms. Warm weather following the first killing frost favors those ear infections, which greatly reduce the vitality of the seed.

A discolored or "stringy" condition of the butt is very suggestive of vascular infection. An extensive survey has failed to consistently correlate a discolored butt with infection of kernels. A discoloration of the butt may occur subsequent to harvesting as the result of poor storage conditions and many types of black molds found established. When the ear is broken in half and a discoloration of the vascular system is observed, it is very good evidence of an infected ear. The growing point consisting of the preformed nodes may be some distance beyond the foci of infection. After mature growth in height is reached the infection may spread so as to become systemic and penetrate the ears. Inoculations under field conditions should easily establish this point.

The pathogenicity of the four parasites discussed has been definitely established under greenhouse conditions. Over 1,500 plants have been grown to determine seedling infection and for inoculation work. It is planned to duplicate much of the inoculation work under field conditions. Nodal and internodal inoculations have indicated the rapid internal development of these parasites. The importance of each of these parasites requires much more study along lines of inoculation and the activity of the fungus.

Severe internal infection of plants even in the tassel stage has not caused any pronounced external symptoms. The corn plant, because

of its morphological structure, is very capable of offsetting root infection without conspicuous external symptoms.

It would hardly seem practical to resort to any means of removing the cap and then disinfecting for control of these rots of corn. Careful selection in the field and proper care in handling and storage along with germination tests should adequately eliminate the possibility of seed corn infection. The question of soil conditions, fertilization, rotation, and cultural methods should be given particular attention in order to reduce infection and secure strong initial growth.

With further inoculation work duplicated under field conditions it is hoped to prove the points which have been discussed theoretically. In pointing out these possibilities it is hoped that other investigators will assist to determine such proof under their conditions.

SUMMARY

(1) Through an extensive survey of field seed corn in Delaware by means of cultural and germination studies at least four prevalent fungous parasites were determined. A brief survey of field corn from 21 other States indicates a similar prevalence of these organisms.

(2) The following four parasites were consistently found in our studies: *Cephalosporium sacchari* Butler; *Fusarium moniliforme* Sheldon; *Gibberella saubinetii* (Mont.) Sacc. and *Diplodia zeae* (Schw.) Lev. The parasitism of these fungi has been determined by inoculations.

(3) So far as we have been able to determine the fungus here referred to as *Cephalosporium sacchari* is reported for the first time as a parasite of corn. *Fusarium moniliforme* is considered identical with *Oospora verticilloides* described on corn in Italy by Saccardo.

(4) Cultural studies of field corn in Delaware show the following prevalence for these parasites: *Cephalosporium sacchari*, 39.54 per cent; *Fusarium moniliforme*, 19.92 per cent; *Gibberella saubinetii*, 5.95 per cent; *Diplodia zeae*, 5.69 per cent.

(5) Inhibition of germination as the result of virulence by these parasites occurs in the following order under cultural and laboratory conditions, *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium sacchari*.

(6) Species of the following genera of fungi have also been found established internal of corn: Aspergillus, Cladosporium, Penicillium, Alternaria, Helminthosporium, Rhizopus, Spicaria, Hormodendrum, Torula, Chaetomium, Colletotrichum, and also several bacteria. These fungi are not considered in the same degree of importance as the above-mentioned parasites. It is probable they have become established as the result of unfavorable field and storage conditions.

(7) The method by which these parasites are carried in the kernel was determined through cultural and histological studies. It was found that the parasites become established in the tissue of the cap and the cavity between the cap and dorsal point of the scutellum. In some instances the fungus works upward underneath the pericarp. Under certain favorable conditions not yet determined the fungus infects and destroys the embryo.

(8) Seed disinfection has not proved successful because of the manner of internal infection.

(9) No uniform symptoms to associate infection of field corn with these parasites can be consistently described. Various discolorations of the germ end of the kernel are found. These symptoms are always

associated with infected seed. Normal-appearing kernels as well as ears may be infected without any external symptoms.

(10) No conclusive evidence was secured which indicates the manner in which infection becomes established in the seed.

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PLATE 1

A.—*Cephalosporium sacchari*, showing typical conidia from host material. The spores are shortly oval to ovoid.

B.—*Cephalosporium sacchari*, showing conidia prior to germination and during early development of germ tube. The conidia commonly become septate prior to germination.

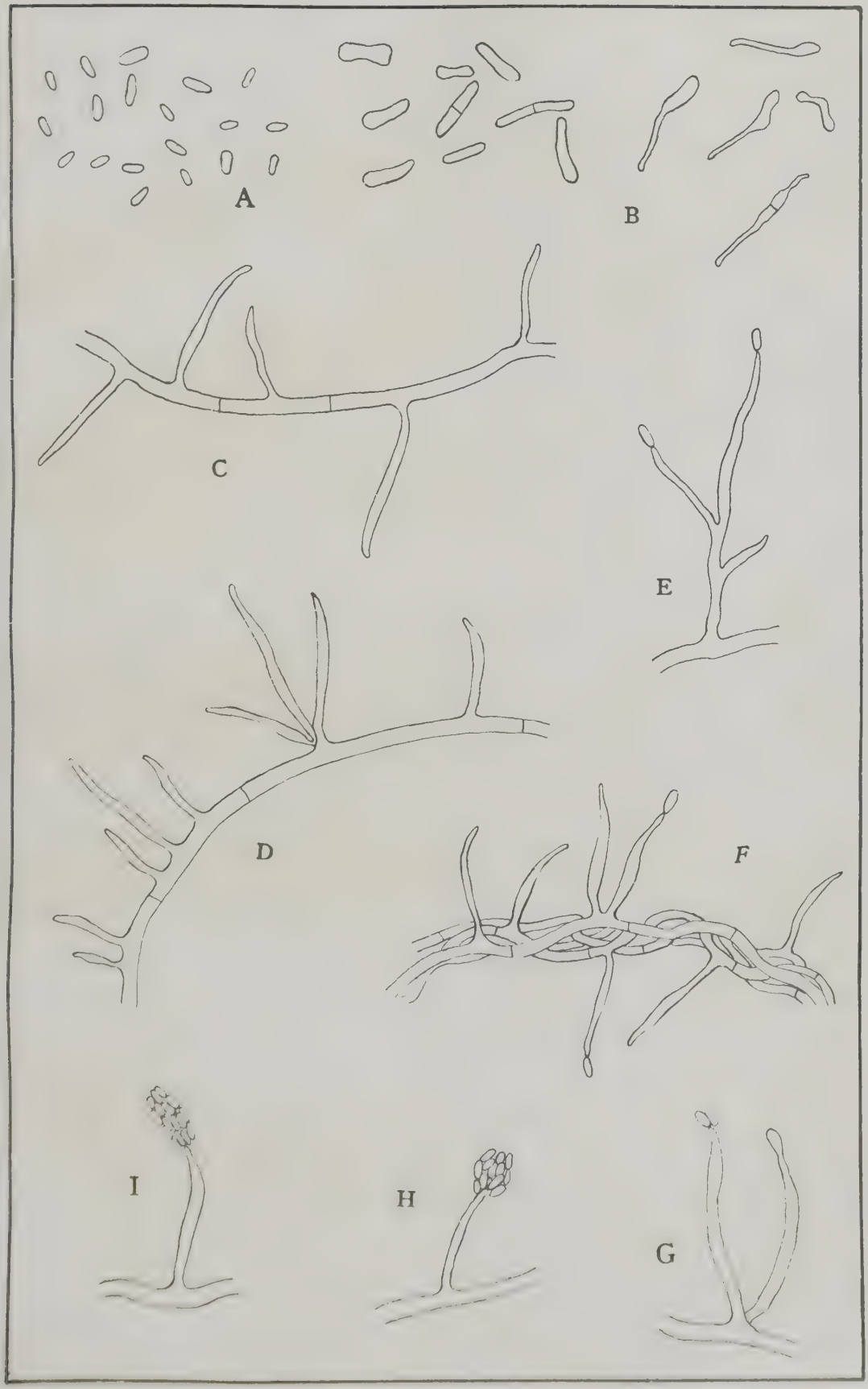
C, D.—*Cephalosporium sacchari*, illustrating the origin of the conidiophores. These are often scattered along the mycelium or a number become grouped as in D.

E.—*Cephalosporium sacchari*, showing a branched conidiophore from growth in culture.

F.—*Cephalosporium sacchari*, showing an intertwined mass of hyphae from a culture. These strands often appear as a definite coremial like growth.

G–I.—*Cephalosporium sacchari*, showing stages in the development of the glomerules. These heads under low power appear similar to those developed by *Fusarium moniliforme*. The spores are not held together in a slime.

Drawn with the aid of the camera lucida, approximately × 600.


A
B
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G
H
I

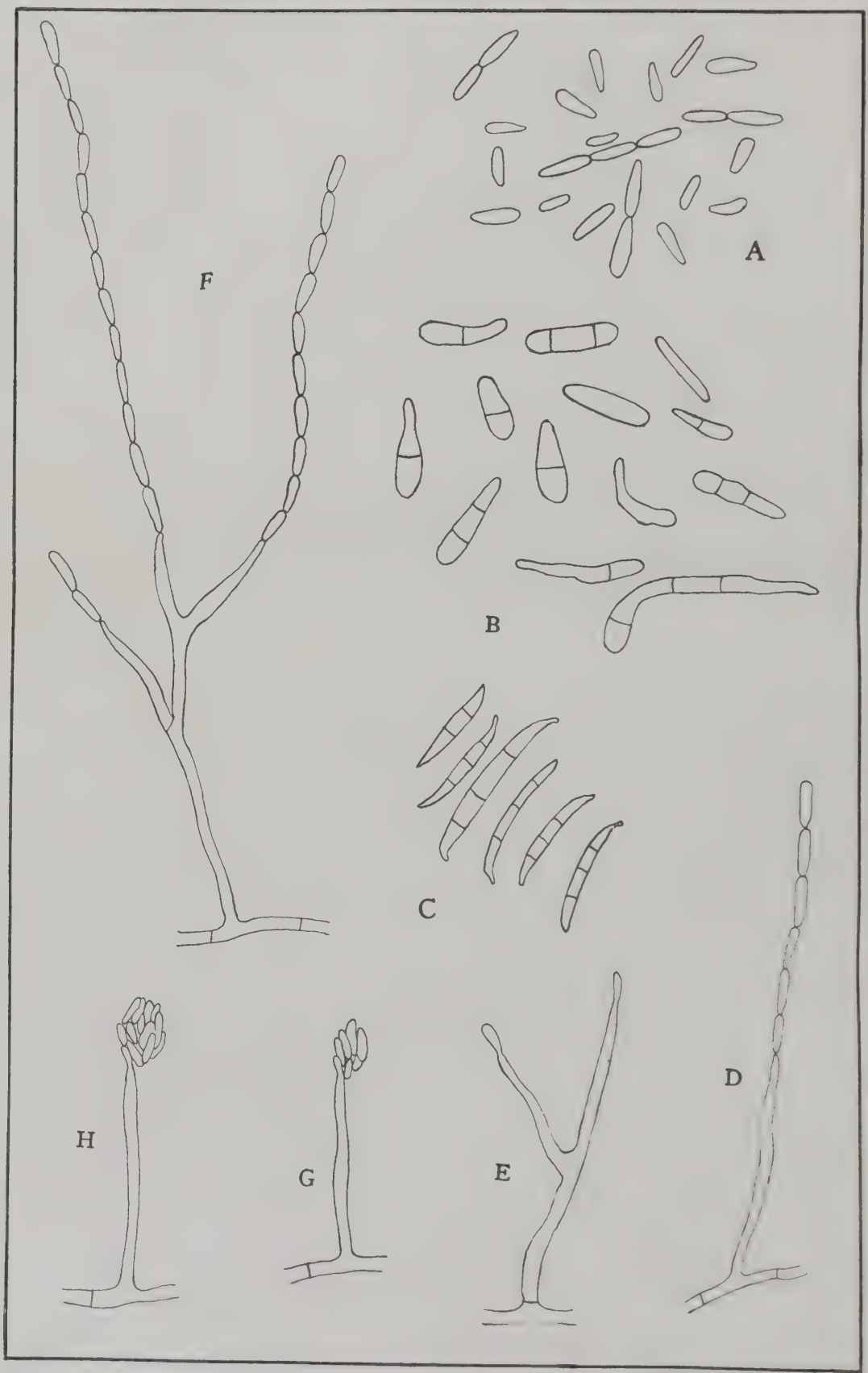
A
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PLATE 2

A.—*Fusarium moniliforme*, showing typical mircroconidia from host material. They are slightly pyriform and the attenuated end of the spore is often sharply muticate.

B.—*Fusarium moniliforme*, showing mircroconidia during early stages of germination. The spores enlarge considerably and often become septate before the germ tube has developed.

C.—*Fusarium moniliforme*, showing typical macroconidia. These were observed to be mostly three septate. They are sparingly developed and were observed in cultures on rice agar and steamed corn meal.

D, E.—*Fusarium moniliforme*, showing simple and branched conidiophores.

F.—*Fusarium moniliforme*, showing a branched conidiophore with typical catenulate microconidia.

G, H.—*Fusarium moniliforme*, showing two simple conidiophores with the microconidia in heads. These heads are commonly found in old cultures but the spores are not held together in a slime.

Drawn with the aid of the cameralucida, approximately ×600.

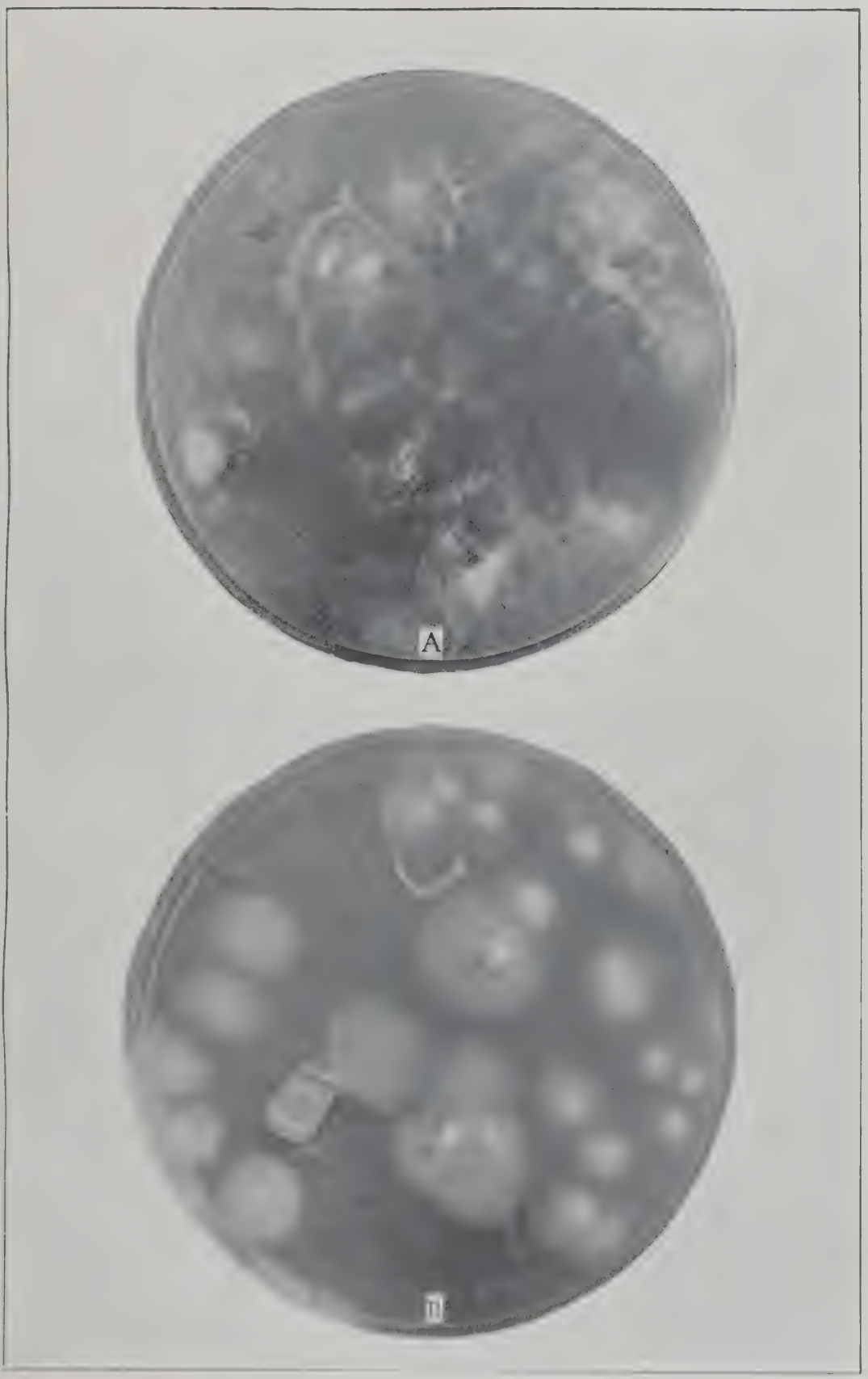
26550—23——3

PLATE 3

A.—Culture of *Fusarium moniliforme* in nutrient dextrose agar 3 weeks old, direct from surface-sterilized kernel from which point was cut off and crushed. The remaining part of the kernel was also left in the culture dish. In submedium a deep lavender is formed.

B.—Culture of *Cephalosporium sacchari* in nutrient dectrose agar, 3 weeks old, direct from surface, sterilized kernel from which point was cut off and crushed. The remaining part of the kernel was also left in the culture dish. The colonies on this medium take on a salmon color.

Somewhat reduced.


A
B

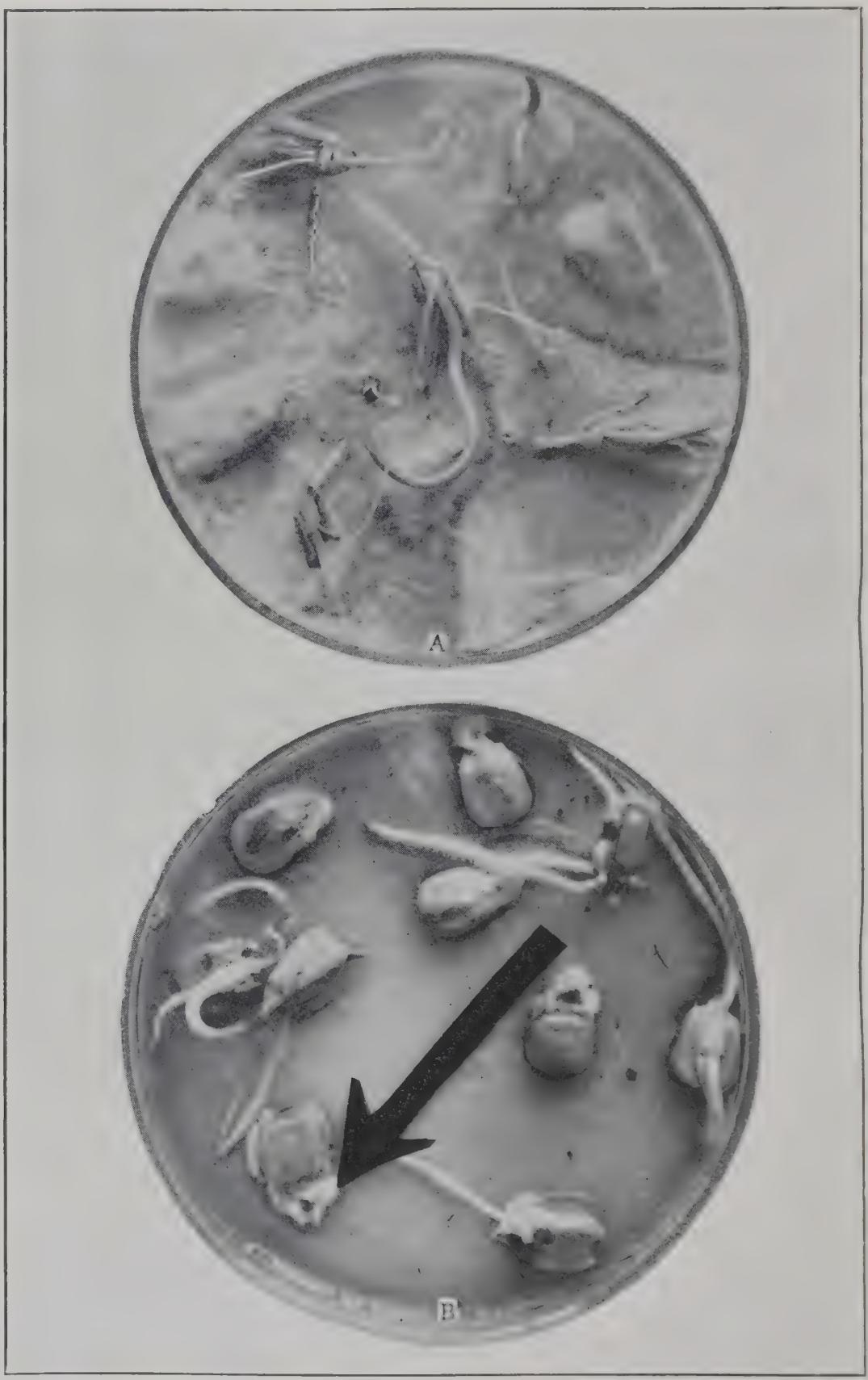

A
B

PLATE 4

A.—Cultures of *Diplodia zeae* coming from surface-sterilized corn germinating in culture dish on 20 cc. of nutrient dextrose agar. Ten kernels are taken from each sample and germinated this way. The effect of the parasite is here shown: 40 per cent of the kernels are dead, 50 per cent are germinating weak, and only 10 per cent are strong.

B.—Cultures of *Cephalosporium sacchari* coming from surface-sterilized corn on nutrient dextrose agar. The arrow points to a culture showing the typical bristle or coremial growth. Some of the kernels are so severely infected that slight retardation of growth is noticeable.

Somewhat reduced.

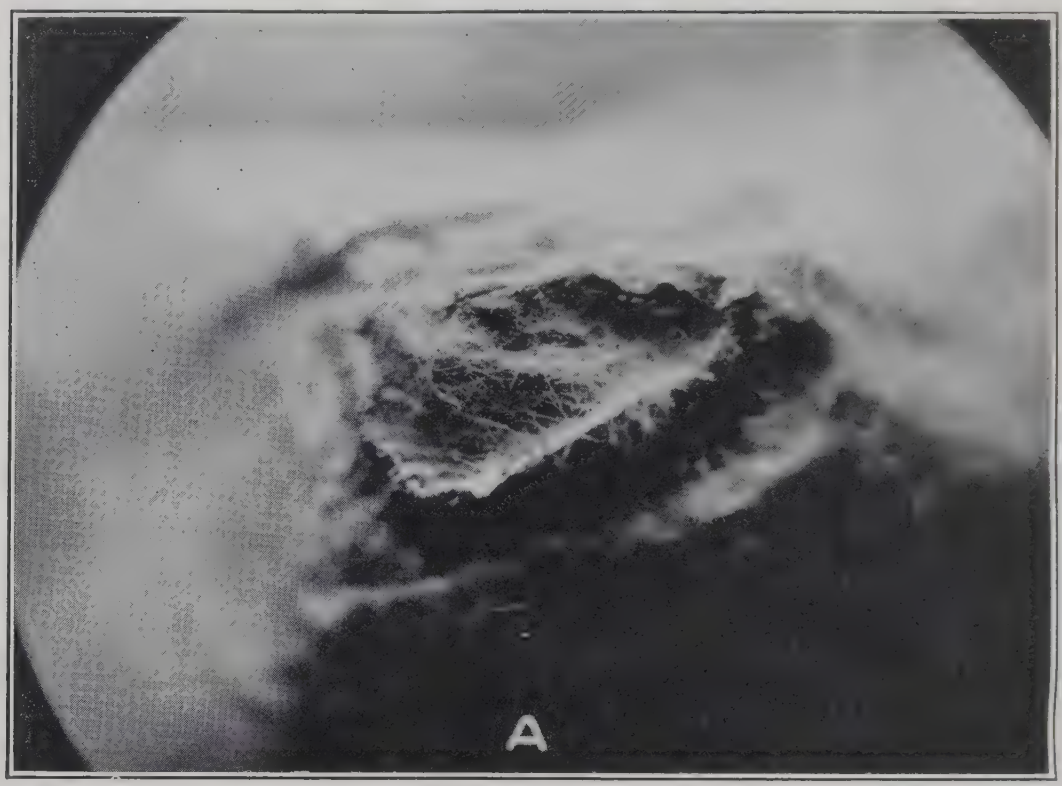
PLATE 5

A.—Sample of corn free from internal paratic fungi germinating on nutrient dextrose agar. The kernels were surface-disinfected with 0.1 per cent bichlorid of mercury in 50 per cent alcohol for one minute, then washed twice in sterile water and immediately placed on the sterile medium.

B.—Sample of corn carrying internally two fungi. The black growth is an *Aspergillus* sp. which is present to the extent of 100 per cent; the white fungus, indicated by the arrow, is *Fusarium moniliforme* which is present to the extent of 40 per cent. The presence of the *Aspergillus* sp. probably indicates that the corn had poor curing conditions.

Somewhat reduced.

A
B


A

Ceph. Saccharo samp #46
Gib. saubinetii Samp 44
Fus. moniliform Samp 45
Diplodia zeae Damp 33
B

PLATE 6

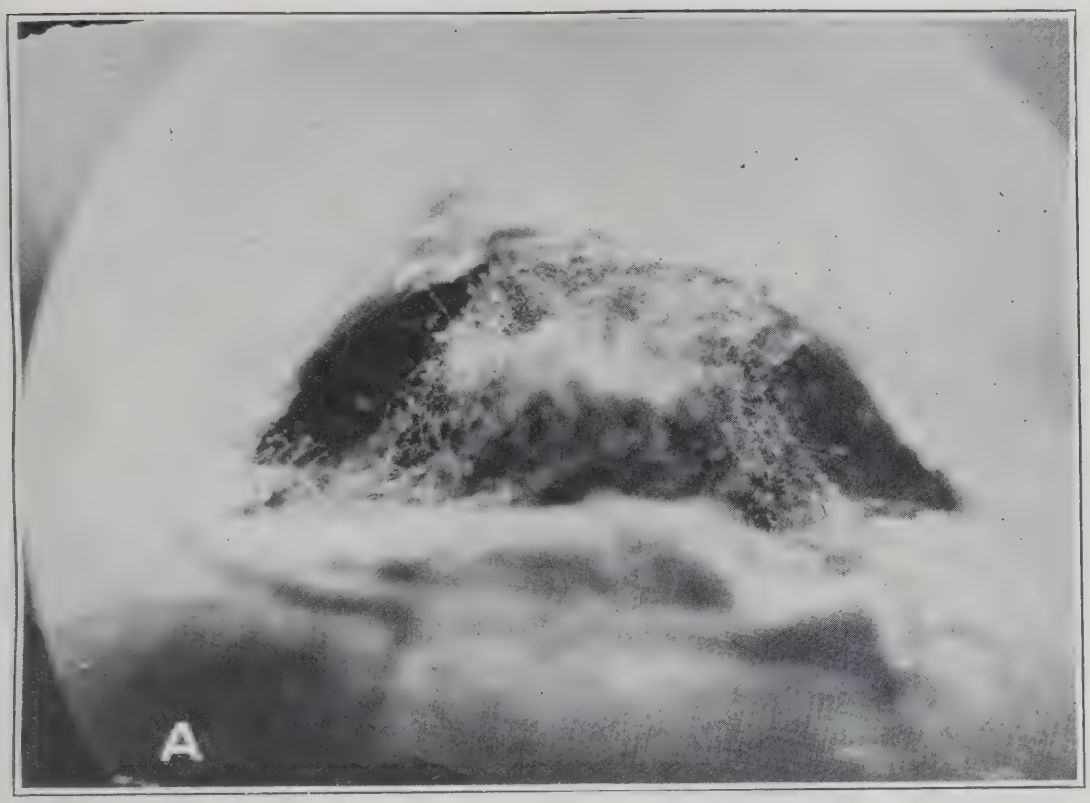
A.—Photomicrograph of tip end of kernel with cap removed, showing the presence of *Diplodea zeae* in the cavity, from sample 33D. Each of the several parasitic fungi here described harbor internal of the kernel in this manner. ×30.

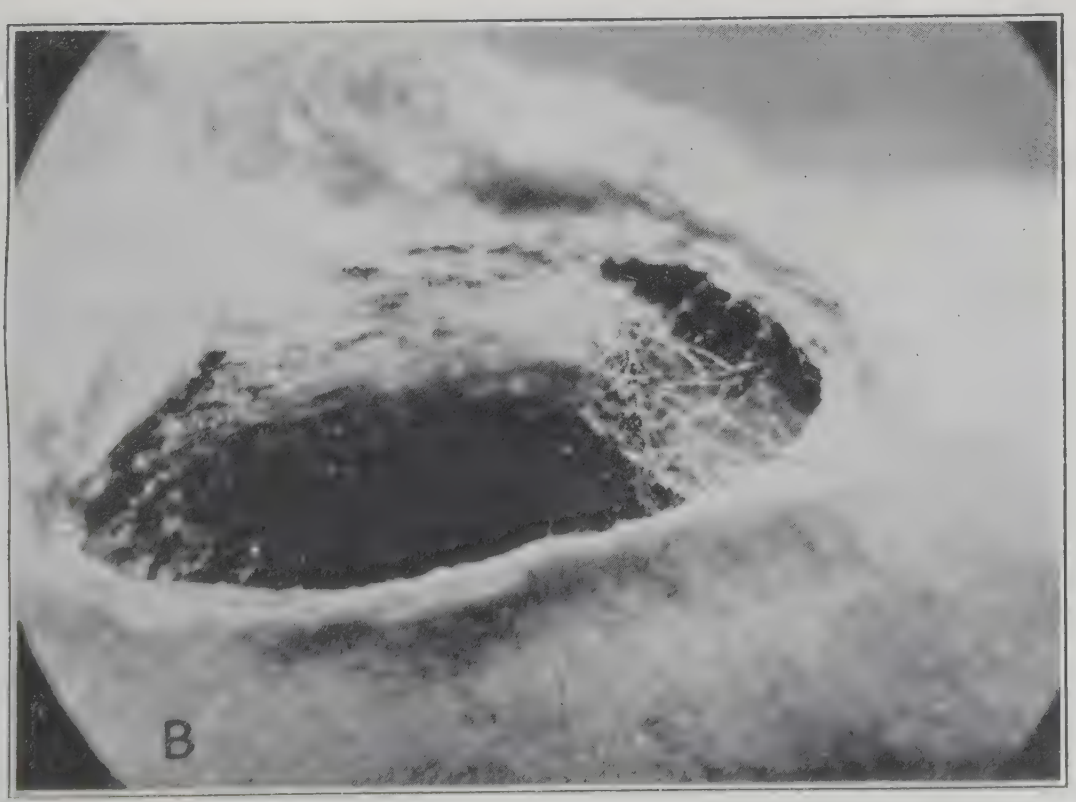
B.—Photograph of mounted kernels with caps removed, ready for taking photomicrographs as illustrated above. Somewhat reduced.

PLATE 7

A.—Photomicrograph of the tip end of kernel with cap removed, showing the presence of *Cephalosporium sacchari* in the cavity. Both *C. sacchari* and *Fusarium moniliforme* fruit (microconidia) are borne abundantly in the cavities under the cap. The thickness of the cap prevents disinfectants from destroying the internal fungus. From sample No. 46. ×30.

B.—Photomicrograph of the tip end of a kernel with cap removed, showing the presence of *Gibberella saubinetii*, from sample 4 A. ×30.


A


B

A

B

PLATE 8

A.—Kernel from sample 321, infected with *Gibberela saubinetii*, in which the parasite has reached the germ and the cavities about the germ. Whenever the germ is invaded by any of the several parasites herein described, germination is either inhibited or greatly retarded. Eighty per cent of the kernels in this sample would not germinate. ×30.

B.—Kernel from Ear 10 B, infected with *Diplodia zeae*, in which the parasite has reached the germ and the cavities about the germ. (See Pl. 9, A.) Seventy per cent of the kernels on this ear were killed by this type of infection; the ear on the surface appeared normal. ×30.

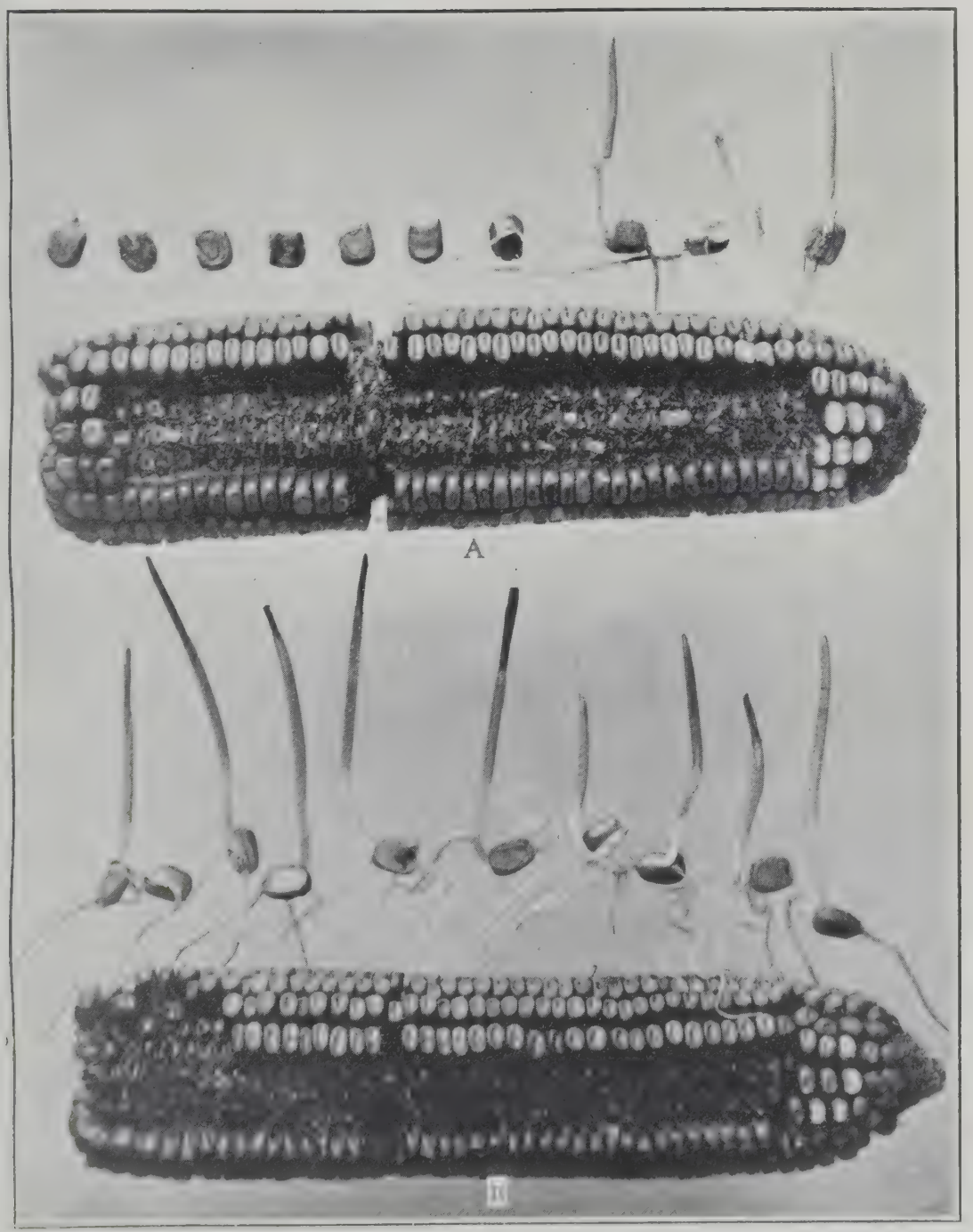
PLATE 9

Two ears of seed corn from the same source. Both ears appeared perfectly normal externally but germination in the modified rag doll showed great difference.

A.—Actual germination of ear 10 B in rag doll; 70 per cent were dead. Culture studies (see Pl. 10) showed 70 per cent of the kernels internally infected with *Diplodia zeae*, 20 per cent with *Gibberella saubinetii*, and 80 per cent with *Cephalosporium sacchari*. *Diplodia zeae* was the active agent in reducing germination.

B.—Actual germination (100 per cent) of ear 10 A, from same source as ear 10 B above. Culture studies showed 100 per cent infection with *Cephalosporium sacchari* and 40 per cent infection with *Gibberella saubinetii*.

Somewhat reduced.


A
B

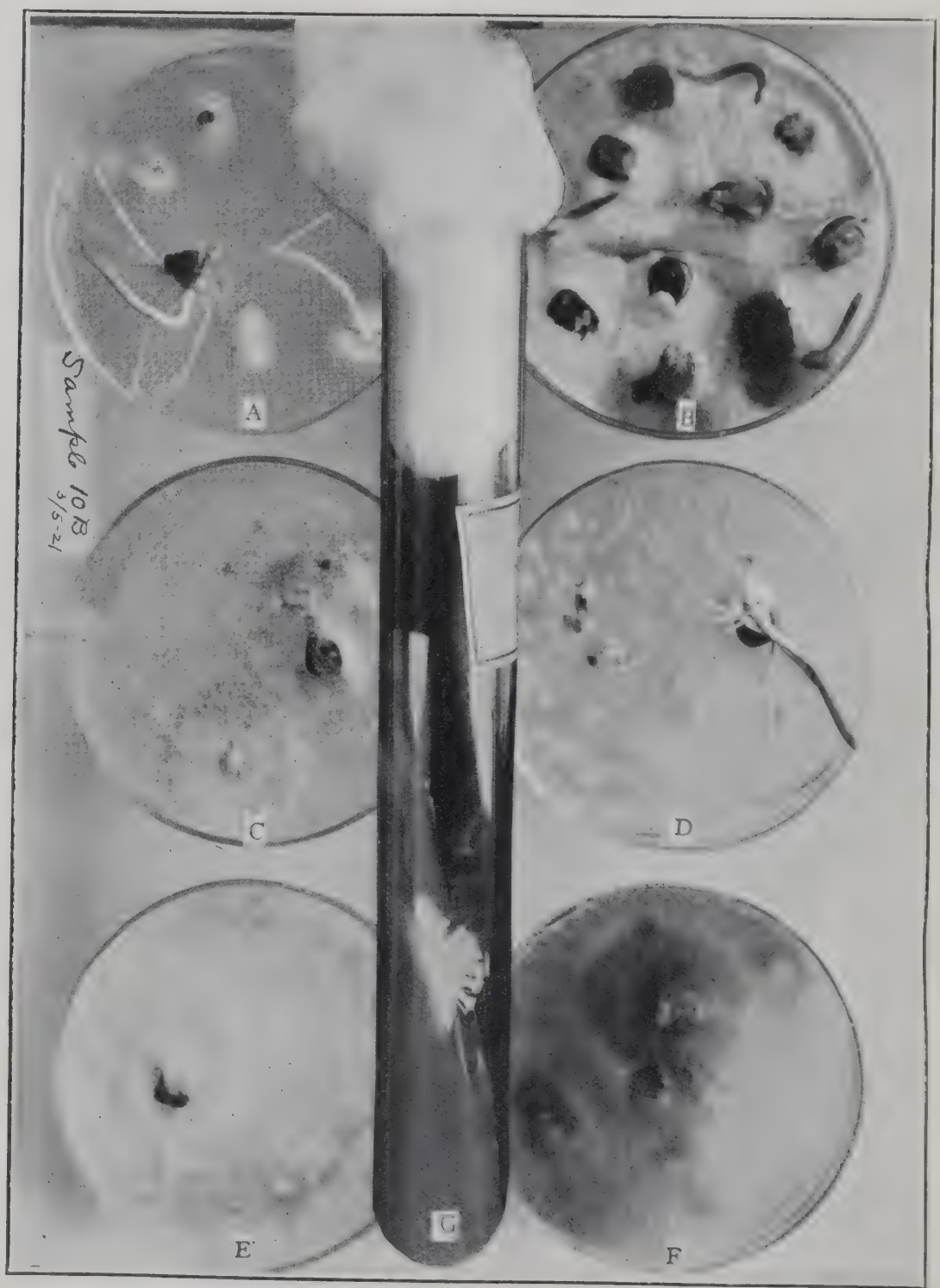

Sample 10 B
3/5-21
A
B
C
D
E
F
G

PLATE 10

Results of the culture method of determining internal fungi in seed corn. Fifteen kernels in each sample were surface-disinfected and washed in sterile water; 10 of these kernels were placed in one dish on 20 cc. of nutrient dextrose agar. Five kernels were placed each in a separate sterile dish, and the points of each of these were cut off, crushed, and plated. The fungi which were internal readiiy developed. The culture work on sample 10 B is here shown, in which 70 per cent of the kernels were infected with *Viplodia zeae* (See cultures in dishes marked B, C, D, and E); 20 per cent of the kernels were infected with *Gibberella saubinetii* (See dish marked F.); 80 per cent of the kernels were infected with *Cephalosporium sacchari* (See dishes marked A, D, and E). In this sample *Diplodia zeae* was the fungus most active in inhibiting germiniation. G, coremial growth produced by *Cephalosporium sacchari*. The coremial growths are not usually so pronounced as this.

Somewhat reduced.

PLATE 11

A.—Seedlings germinated in modified rag doll from kernels of sample 181–c.

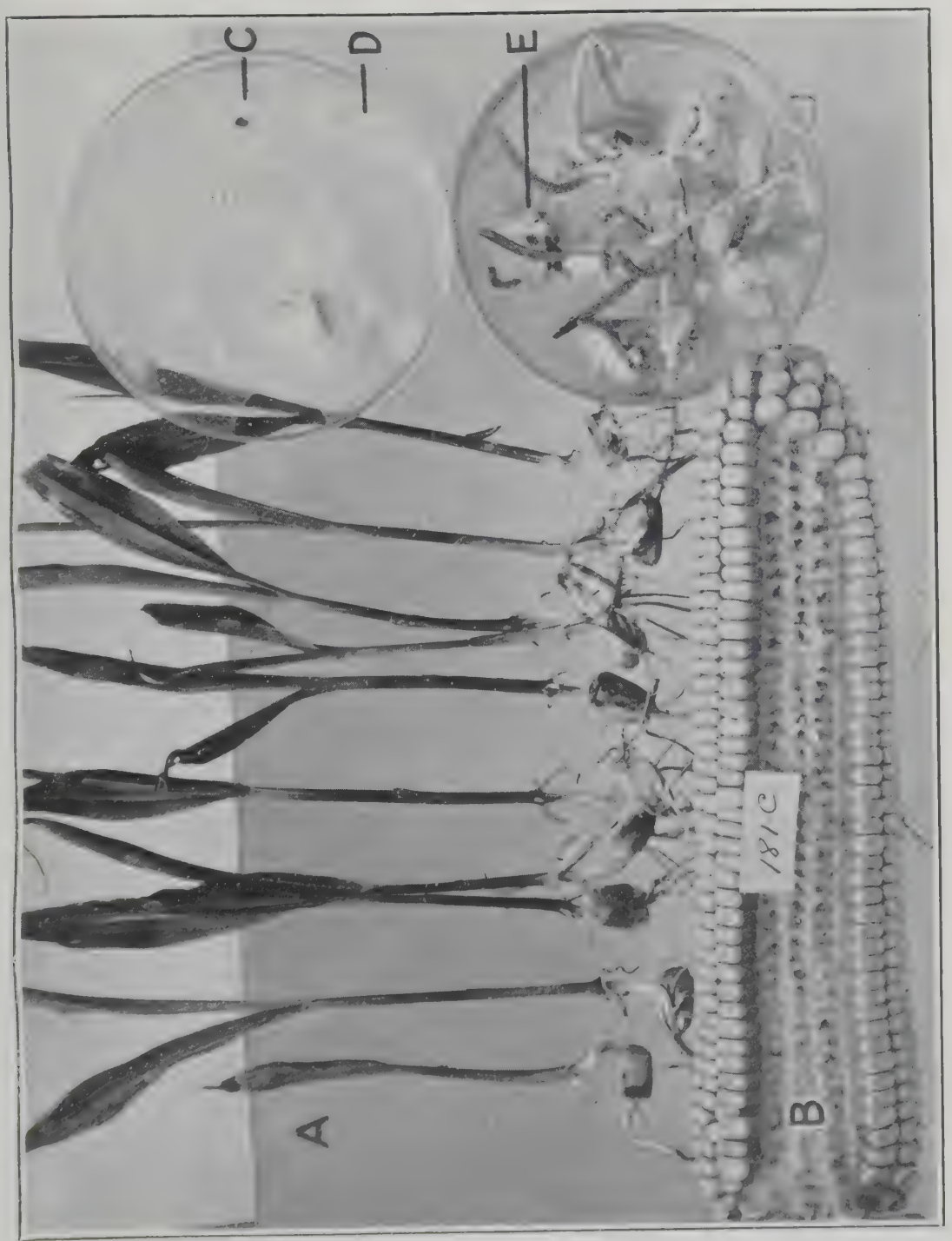
B.—Sample 181–c. This sample carried internal infection with *Fusarium moniliforme* to the extent of 100 per cent and *Cephalosporium sacchari* 100 per cent and yet gave 100 per cent germination and very strong seedlings in the rag doll. Injury and retardation of germination take place only when the parasitic fungi come in contact with the germ of germinating seedlings.

C.—Culture of *F. moniliforme*, internal of same kernel as D.

D.—Culture of *C. sacchari*, internal of same kernel as C.

E.—Ten surface-sterilized kernels placed on 20 cc. of nutrient dextrose agar, all showing both *F. moniliforme* and *C. sacchari*. Only one kernel failed to germinate in the culture dish; 100 per cent gave strong germination in the modified rag-doll germinator.

Somewhat reduced.


C
D
E
A
B
181 C

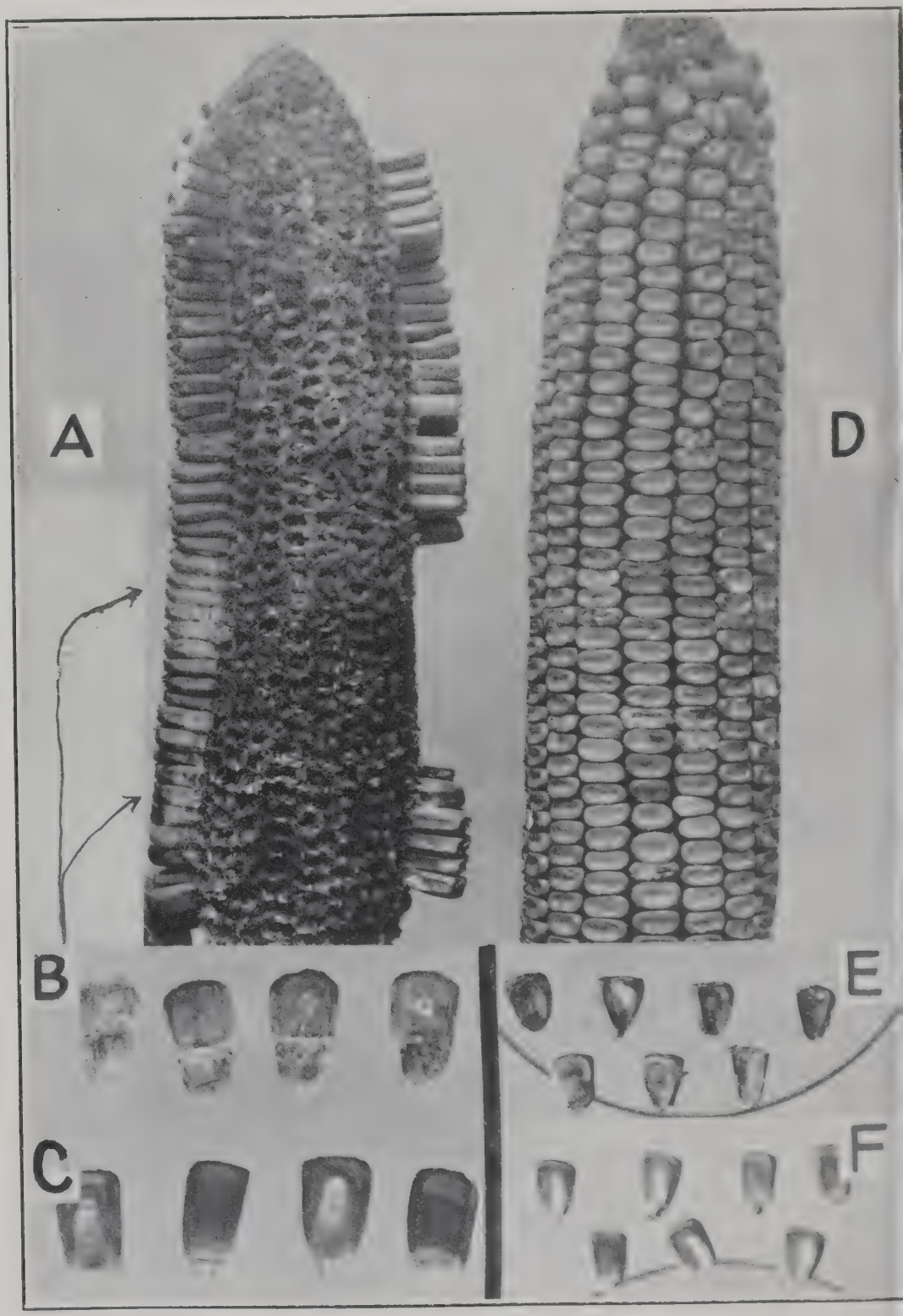

A
D
B
E
C
F

PLATE 12

A.—Ear affected with kernel crack at areas indicated by the arrow.

B.—Injured kernels from ear shown in A.

C.—Uninjured kernels from ear shown in A.

More than 60 per cent of the kernels on the same ear were not injured, as indicated in C. Some of the cracked kernels will germinate. Associated with this cracking are often found *Fusarium moniliforme*, *Cephalosporium sacchari*, and occasionally a *Spicaria* sp. The cause of the cracking is yet to be determined.

D.—Ear affected with a typical kernel-rot caused by *F. moniliforme*.

E.—Rotted kernels from ears shown in D. Such rotted kernels will not germinate. Associated with such rotting is often the lavender discolorations characteristic of the growth of *F. moniliforme*.

F.—Healthy kernels from ear shown in D.

Somewhat reduced.

PLATE 13

Typical ear rot resulting from natural infection with *Gibberella saubinetii*. In th type of rot it seems probable that infection takes place through the silk mass in th late milk or dough stage. Cultures of the kernels near the base or butt of ear A, gav no evidence of *Gibberella saubinetii* internal, possibly indicating that infection wa from above.

Somewhat reduced.

A
B

COTTON ROOTROT IN ARIZONA[1]

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It has been generally accepted that the fungus, *Ozonium omnivorum*, described by Pammel[2] in 1889, is the cause of the disease known as Texas rootrot, but definite proof has been lacking. The records available show that but little has been added to the knowledge of the life history of the fungus since it was first studied by Pammel,[2] a notable exception being the work of Duggar[3] in 1916, who described a conidial stage from material which he found in a cotton field near Paris, Tex., in 1915. However, Duggar's classification, based on the character of this fruiting form, does not appear to have been adopted by other pathologists.

The conditions at Sacaton, Ariz., during the seasons of 1921 and 1922 permitted unusually satisfactory observations upon the intimate relationship existing between the disease and a fruiting form of the fungus, which is evidently identical with that described by Duggar. In the Salt River Valley, Ariz., during the seasons 1917 to 1919 the fruiting fungus had been observed on several occasions, sometimes in abundance following a rainy period and often as isolated spots where a depression or crack in the soil afforded favorable conditions; but at no time was the material so abundant and so definitely associated with the presence of the disease as at Sacaton during the past two seasons.

In comparing the manner of spread of the disease in various crops, it was noted that in alfalfa fields its behavior was very suggestive of fairy rings. The perfectly formed circles, consisting of an outer ring of recently dead plants, an inner ring, or "bare zone," where only the old stubble of dead plants remains, and a central zone occupied by reestablished plants arising from fragments of crowns or roots not fully destroyed, establish the fact that the disease spreads from a center in ever-widening circles and that having passed a given place leaves this spot free from the disease until reinfected. In badly infected fields where the disease is of long standing the crossing and recrossing of the widening rings may completely obscure the fairy-ring effect.

The resemblance to fairy rings is made still more striking by the abundant crops of fruiting bodies, which in Arizona appear on the periphery of the circles shortly after the occurrence of rainy weather. The newly formed fruiting bodies appear as feltlike mats on the surface of the ground, or in cracks or depressions, and are rarely found more than 6 or 8 inches from the outer circle of recently wilted plants. At times they have been so abundant as to cover over 300 square feet of soil surface in a 22-acre alfalfa field where 3 or 4 acres of the plants had died.

[1] Accepted for publication Dec. 16, 1922.

[2] PAMMEL, L. H. COTTON ROOT-ROT. *In* Tex. Agr. Exp. Sta. 2d Ann. Rpt. 1889, p. 61–86, 3 pl. 1890.

[3] DUGGAR, B. M. THE TEXAS ROOT-ROT FUNGUS AND ITS CONIDIAL STAGE. *In* Mo. Bot. Gard. Bul., v. 3, p. 11–23, 5 fig. 1916. Bibliography, p. 23.

CONTROL EXPERIMENTS

The characteristic behavior, indicating that the disease is virulent only near the rim of the circles, encouraged the writer's attempted use of certain fungicides in the effort to check the spread of the fungus. By thoroughly saturating the soil to a depth of 4 feet with a solution of formaldehyde (1 part 40 per cent formalin to 100 parts water), in small circular areas where the activity had just started, it was found possible to prevent further spread of the disease. It appears that the fungus mycelium must extend a foot or more in advance of the last plant showing distress, since to be effective the soil 18 inches outside the apparent periphery in alfalfa fields must also be included in the treatment, it was found.

With cotton plants the mycelium appears to extend for at least 2½ feet in advance of the ring of recently wilted ones, there being instances where the disease reappeared after treatment on plants 2½ feet away. In no case in cotton fields has the disease reappeared where the treated area extended as far as 3 feet outside the ring of recently wilted plants. The most practical method of applying the solution seems to be that of throwing up a small dike around the area to be treated and pouring in the solution gradually until the soil has been saturated to a depth of at least 4 feet.

None of the areas in alfalfa fields treated as described above in July and early August, 1922, have shown further disease activity after 60 days. With the control areas where dikes were thrown up, but no fungicide applied, the disease has progressed from 4 to 4½ feet during that time. There has been no reappearance of the disease outside any of the areas in cotton fields during 50 days after treatment. In the control areas the disease has advanced from 6 to 8 feet during that interval.

The first successful attempt to communicate the rootrot disease to healthy cotton plants was made in August, 1922. The inoculation experiments were conducted with normal plants in the field, since it had been found difficult to grow normal cotton plants or develop them into a fruiting condition in pots in the greenhouse under the climatic conditions which prevail at Sacaton in summer. An area was selected on which there had been no evidence of rootrot since the experiment station was established in 1907. A trench was made on July 29, approximately 2 feet wide, 9 feet long, and 2½ feet deep, between two cotton rows in which the plants were normal in every respect and fruiting heavily. With a small hand pick the soil on one side of the trench was dug away from the roots of each plant alongside the trench until 15 inches or more of each tap root had been exposed in the small vertical channels. On this side of the trench sections of rootrot-diseased cotton roots 1 inch in length, previously washed in distilled water, were inserted in the channels and placed end to end and in direct contact with the healthy tap root. The full length of the channel was then stuffed with sterile cotton soaked in distilled water, and then soil, which had been saturated with water, was applied with a trowel on top of the cotton until the channels were completely filled.

On the other side of the trench the roots were exposed in the same manner, but instead of diseased roots being used the cotton wrapping was soaked in distilled water containing 50 cc. per liter of fresh spores collected from active rootrot spots. After 21 days following a rainy period, one of the plants treated with diseased roots wilted suddenly and upon

being pulled up showed in abundance the characteristic mycelium of the rootrot fungus. Within 5 days four other inoculated plants died in the same characteristic manner, but by this time the soil on the sides of the trench had become so depleted of moisture that the disease was unable to make further progress.

From the first inoculated plant which died (it was pulled up immediately after wilting), the mycelium was isolated and pure cultures were prepared after the method of Atkinson.[4] Bits of sterile cotton roots were used as the medium. After abundant growth had been obtained from one of these pure cultures on a layer of sterile cotton roots in moist chambers, the material was conveyed to another trench that had been prepared a few feet from the first. This material was then used to inoculate 10 plants on one side of the trench, 4 plants being left as a control. The 14 plants on the other side of the trench were treated with diseased roots as described above. The plants were kept well supplied with moisture and in 12 days they began dying almost simultaneously on both sides of the trench. By September 20, the disease had appeared in all but 4 or 5 of the plants on both sides of the trench, including the four control plants, which had been attacked one at a time by the invasion of the mycelium through the soil from the inoculated plants. By October 1, the mycelium had spread from the pure-culture treatment to the plants 3½ feet away in the next row, and 4 of them had succumbed by this date. After seven weeks none of the plants which had been treated with spores showed any signs of distress.

Spores collected from fresh fruiting mats were induced to germinate in distilled water and in artificial media after several days, but the resulting growth was extremely slow. After five weeks in the Van Tieghem cells the germ tubes have grown but two or three times longer than the diameter of the spores. The difficulty in inducing the spores to develop in artificial media and the failure to induce the disease by applying them to the roots of healthy plants is not surprising. If the conditions necessary for the development of spores were not very exacting, the enormous quantities in which they are produced would long since have made the distribution of the disease universal.

It is of the utmost importance to determine as promptly as possible to what extent the promising results obtained in these local experiments are applicable in other regions.

SUMMARY

(1) At Sacaton, the fungus causing rootrot of cotton and alfalfa fruits abundantly in favorable seasons, the fruiting masses being identical apparently with those described by Duggar.[5]

(2) The fungus spreads from a center in ever-widening circles after the manner of a fairy-ring fungus.

(3) Plants have been inoculated successfully with pure cultures of the mycelium.

(4) Spores are germinated with difficulty and have not been used successfully as a medium of inoculation.

(5) The spread of the disease may be checked by the use of formaldehyde.

[4] ATKINSON, G. F. METHOD FOR OBTAINING PURE CULTURES OF FAMMEL'S FUNGUS OF TEXAS ROOT ROT OF COTTON. *In* Bot. Gaz., v. 18, p. 16–19. 1893.

[5] DUGGAR, B. M. OP. CIT.

CORRELATIONS BETWEEN VARIOUS CHARACTERS OF WHEAT AND FLOUR AS DETERMINED FROM PUBLISHED DATA FROM CHEMICAL, MILLING, AND BAKING TESTS OF A NUMBER OF AMERICAN WHEATS [1]

By JACOB ZINN [2]

Formerly [3] Associate Biologist, Maine Agricultural Experiment Station

INTRODUCTION

From a plant breeder's point of view it would be very desirable to establish an index by means of which the baking quality or strength of wheat flour could be determined at an early stage of the breeding work so that the weak wheat strains could then be discarded, instead of carrying them through all the stages of propagation and the processes in the bakehouse. The solution of this problem calls for the establishment of a definite relation between a wheat character, determinable early in the selection or breeding work, and the strength of the flour as measured by the loaf volume.

A study of the literature on the chemistry of wheat (*18*)[4] reveals a marked divergence of views as to the significance of the various chemical characteristics of wheat in relation to the bread-making quality of the flour. Almost without exception, the work on the relationship of the chemical characters to bread-making value is characterized by the lack of statistical methods. The writer is aware of no publication in which this relationship and its degree are expressed in terms which will allow of comparison.

In the present paper a large number of published data on the chemistry of wheat are compiled and the coefficients of correlation for the important chemical characters computed. In submitting the results of this study attention must be called to the limitations of the material. In order to exclude possible errors due to differences in the nature of the material and in technic, it was thought advisable to an alyze the data into groups by States and according to the kind of wheat rather than to lump them together into larger classes including several States or wheat districts. This, of course, resulted in a reduction of the data in the individual groups, which in some correlation tables reaches a point where conclusions can not always safely be drawn.

A further limitation of the material considered in this study lies in the relatively small amount of data on the chemistry of wheats and flours derived from pure strains. The data on pure lines are limited both in regard to localities and number of samples analyzed. The writer believes that the proper approach to the solution of the question of correlations

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[1] Accepted for publication Nov. 14, 1921.

Wheat Investigations II. Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 147.

[2] In the preparation of this paper the writer received helpful suggestions from Dr. John W. Gowen, of the Maine Agricultural Experiment Station, and assistance from Mr. H. C. McPhee and Mr. E. R. Ring, also of the Maine station, in the tabulation and computation of the data.

[3] Dr. Jacob Zinn died in October, 1921.

[4] Reference is made by number (italic) to "Literature cited," p. 548.

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between the characters here considered must be based upon a large amount of data secured from analyses of pure lines and made by the same laboratory, rather than any analyses of commercial varieties. In the light of this proposition the present paper is to be regarded as a preliminary study, as the amount of data from pure lines here included is very limited.

Notwithstanding these limitations the present study is of some interest. It represents the first attempt to analyze statistically a large amount of raw data by computing from them correlation coefficients for a number of characters. It should throw some light upon the relationships of the chemical characters where formerly they were based merely upon observation or inspection of data. It should also be instructive to compare, in the future, the results obtained in the main from commercial varieties with those to be secured from pure lines of wheat. Finally, it may furnish suggestions of service in appraising the bread-making value of wheats.

MATERIAL AND METHODS

The material used in the present study is the data from chemical, milling, and baking tests published by the agricultural experiment stations of Colorado (*7*), Idaho (*8*), Kansas (*16*), Maine (*18*), Minnesota (*2*, *3*, *4*, *5*), Montana (*13*), North Dakota (*6*, *9*, *10*, *15*), Ohio (*1*, *17*), Wisconsin (*11*), and the Central Experimental Farms of Canada at Ontario (*12*). While the choice of the material was naturally limited by the availability or lack of data for the different grain-growing regions, an inspection of the localities stated above will show that they include the important districts of the hard red spring and semihard red spring wheats, the hard red winter, and semihard red winter wheats. No data are included on the white soft wheats of the Pacific coast, the soft winter wheats of the Mississippi district, and the durum wheats.

The classification of the material is first based upon the geographic distribution of the wheats and flours, the data of each experiment station being treated separately. The data of each experiment station are further subdivided according to the kind of wheat, the spring wheats and winter wheats being grouped separately. A further subdivision of the material was effected by grouping the spring wheats and winter wheats according to whether they are commercial varieties or pure strains.

The coefficients of correlation computed in this study relate to 10 important values secured from chemical analysis and milling and baking tests. Protein content in wheat is correlated with protein content in the flour, dry gluten, gliadin, and yield of flour. Loaf volume is correlated with protein content in wheat, protein content in the flour, dry gluten content, wet gluten content, quality of gluten, gliadin water absorption, and yield of flour. Dry gluten is correlated with gliadin and water absorption. As already noted, the whole material was analyzed into four groups: Spring wheats, winter wheats, commercial varieties, and pure strains. Of the total number of 100 computed coefficients of correlation, 30 relate to commercial varieties of spring wheat, 27 to pure strains of spring wheat, 32 to commercial varieties of winter wheat, and 11 to pure strains of winter wheat.

Since the data used in this paper have all been published by the experiment stations enumerated above, it is not necessary to publish the large number of correlation tables. However, for each kind of correlation, with a few exceptions, a representative correlation table is here

published, giving the frequency distribution typical for each pair of characters. Following each table are tabulated summaries of correlation coefficients pertaining to the characters correlated in the preceding table. The calculation of the coefficients of correlation was carried to the sixth decimal place and recorded to the fourth.

ANALYSIS OF DATA

PROTEIN CONTENT IN WHEAT AND FLOUR

From the nature of these two values a very close relation between them may be anticipated. The degree of that relationship is of interest.

TABLE I.—*Correlation between protein content in the wheat and the flour of Minnesota spring wheats of commercial varieties*

Protein in the wheat (per cent).	Protein in the flour (per cent).												Total
	9.00 to 9.50	9.50	10.00	10.50	11.00	11.50	12.00	12.50	13.00	13.50	14.00	14.50 to 15.50	
9.5 to 10.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....
10.00	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1
10.50	1	6	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	9
11.00	3	4	6	4	.....	.....	.....	.....	.....	.....	.....	.....	17
11.50	.....	.....	10	10	3	2	.....	.....	.....	.....	.....	.....	25
12.00	.....	.....	.....	11	21	6	1	.....	.....	.....	.....	.....	39
12.50	.....	.....	2	2	13	17	1	1	.....	.....	.....	.....	36
13.00	.....	.....	.....	2	1	7	9	1	1	.....	.....	.....	21
13.50	.....	.....	.....	.....	.....	3	15	6	4	.....	1	.....	29
14.00	.....	.....	.....	.....	.....	1	1	3	7	1	.....	.....	13
14.50	.....	.....	.....	.....	.....	.....	.....	.....	3	2	2	1	8
15.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	1	4
15.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	3	1	1	5
16.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	1
16 to 16.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	....
Total ..	4	11	20	29	38	36	27	11	15	7	7	3	208

$r = 0.9120 \pm 0.0079$.

TABLE II.—*Correlation coefficients for protein content in the wheat and the flour*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
Colorado spring wheats	48	0.8036±0.0344
Minnesota spring wheats	208	.9120± .0079
North Dakota spring wheats	119	.9161± .0100
Montana spring wheats	34	.9710± .0066
Kansas winter wheats	47	.8957± .0194
Minnesota winter wheats	43	.9216± .0154
Montana winter wheats	91	.9259± .0101
Ohio winter wheats	99	.9438± .0074
Idaho winter wheats	60	.9728± .0046
Pure strains:		
Minnesota spring wheats	48	.8921± .0199
North Dakota spring wheats	28	.9686± .0078
Wisconsin winter wheats	7	.8814± .0571
Ohio winter wheats	25	.9148± .0220

The data in Tables I and II show a very high positive correlation between protein content in wheat and flour. The coefficients of correlation range from 0.8036 ± 0.0344 for the Colorado spring wheats to 0.9728 ± 0.0046 for the Idaho winter wheats. The average for the commercial varieties is almost identical with that for the pure strains.

DRY GLUTEN AND PROTEIN IN THE WHEAT

In view of the significance attached to the gluten content as influencing the strength of flour, the relation of the former to the protein content in the wheat is of interest.

TABLE III.—*Correlation between dry gluten and protein content in Ohio winter wheats of commercial varieties*

Dry gluten content (per cent).	Protein in the wheat (per cent).									Total.
	8.50 to 9.00	9.00	9.50	10.00	10.50	11.00	11.50	12.00	12.50 to 13.00	
6.50 to 7.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7.00	4	1	.....	.....	.....	.....	.....	.....	.....	5
7.50	1	2	2	.....	.....	.....	.....	.....	.....	5
8.00	.....	4	2	.....	1	1	.....	.....	.....	8
8.50	.....	.....	2	3	3	1	.....	.....	.....	9
9.00	.....	.....	.....	1	3	3	3	.....	.....	10
9.50	.....	.....	.....	.....	.....	1	4	2	.....	7
10.00	.....	.....	.....	.....	.....	3	3	1	.....	7
10.50	.....	.....	.....	.....	.....	.....	3	1	1	5
11.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12.00	.....	.....	.....	.....	.....	.....	.....	.....	1	1
12.50	.....	.....	.....	.....	.....	.....	.....	1	.....	1
13.00 to 13.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	5	7	6	4	7	9	13	5	2	58

$r = 0.8695 \pm 0.0217$.

TABLE IV.—*Correlation coefficients for characters dry gluten and protein content in wheat*

Kind of wheat.	Number of samples.	Coefficients of correlation.
Commercial varieties:		
Colorado spring wheats	48	0.6951 ± 0.0503
North Dakota spring wheats	37	$.8300 \pm .0345$
Ohio winter wheats	58	$.8695 \pm .0217$
Idaho winter wheats	60	$.9824 \pm .0031$
Pure strains:		
Maine spring wheats	31	$.9603 \pm .0095$
Ohio winter wheats	13	$.9483 \pm 0.0190$

The correlation between these two chemical characters is positive and very high, as would be expected. The correlation coefficients range from 0.4951 ± 0.0503 for the Colorado spring wheats to 0.9824 ± 0.0031 for the Idaho winter wheats, the data for the two States marking again the limits of the range as in the case of the relationship of the protein in wheat to protein in the flour.

GLIADIN AND PROTEIN CONTENT IN THE WHEAT

Gliadin, both its absolute amount in the flour and in the form of the gliadin number (ratio of gliadin to glutenin) has by some chemists been regarded as the determining factor of strength of the wheat flour. The degree of its association with the crude protein content of wheat should, therefore, be a matter of interest in the study of the chemical relationships in wheat.

TABLE V.—*Correlation between gliadin and protein content in North Dakota spring wheats of commercial varieties*

Gliadin (per cent).	Protein in wheat (per cent).																					Total.
	9.00 to 9.50	9.50	10.00	10.50	11.00	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	17.00	17.50	18.00	18.50 to 19.00	
3.50 to 4.00																					....
4.00													1								1
4.50	1												1								2
5.00					1	1															2
5.50		1	2	1	1	2	1	1		1											10
6.00						1	4	3	2												10
6.50							2	7	3	2		2									16
7.00										5	2	4	3	1		3	1				19
7.50										4	6	2	2	3				1			18
8.00												2	3	2		2				1	10
8.50												4	1	3	2	5			1		16
9.00															1		2	2			5
9.50																1	1		1	1	4
10.00 to 10.50																					....
Total	1	1	2	1	2	4	7	11	5	12	8	14	11	9	3	11	4	3	2	2	113

$r = 0.7863 \pm 0.0243$.

TABLE VI.—*Correlation coefficients for gliadin and protein in wheat*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	113	0.7863 ± 0.0243
Kansas winter wheats	37	.8508 ± .0306
Ohio winter wheats	40	.9244 ± .0160
Pure strains:		
North Dakota spring wheats	26	.9295 ± .0181

The data in Tables V and VI show a very close correlation between gliadin and crude protein in wheat. The correlation coefficients range from 0.7863 ± 0.0243 for the North Dakota commercial varieties of spring wheat to 0.9295 ± 0.0181 for the North Dakota pure strains of spring wheat.

The characters so far discussed are all component parts of the crude protein of wheat, and their mutual relationship is to be *a priori* assumed. It is of interest, however, to note the intensity of this relationship as given by the correlation coefficients.

FLOUR YIELD AND PROTEIN IN WHEAT

The question of relation between flour yield and protein in the wheat is of concern especially to the miller.

TABLE VII.—*Correlation between flour yield and protein in Minnesota spring wheats of commercial varieties*

Protein in wheat (per cent).	Yield of flour (per cent).												Total.
	65 to 66	66	67	68	69	70	71	72	73	74	75	76 to 77	
9.50 to 10.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10.00	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1
10.50	1	.....	.....	1	1	3	1	1	1	.....	.....	.....	9
11.00	1	.....	1	2	3	6	.....	3	1	.....	.....	.....	17
11.50	1	.....	3	.....	3	5	5	.....	7	.....	1	.....	25
12.00	.....	1	1	.....	3	6	10	8	6	1	3	.....	39
12.50	.....	1	.....	.....	4	7	6	7	3	3	.....	2	33
13.00	.....	.....	1	1	1	3	3	3	6	1	1	.....	20
13.50	.....	.....	1	1	3	2	5	4	11	1	.....	.....	28
14.00	.....	1	1	.....	.....	3	1	1	5	.....	.....	.....	12
14.50	.....	.....	.....	.....	2	2	.....	.....	3	1	.....	.....	8
15.00	.....	.....	.....	.....	2	1	.....	.....	.....	1	.....	.....	4
15.50	.....	.....	.....	.....	2	.....	.....	.....	3	.....	.....	.....	5
16.00	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	1
16.50 to 17.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	3	3	8	5	24	38	32	27	47	8	5	2	202

$r = 0.1789 \pm 0.0459$.

TABLE VIII.—*Correlation coefficients for protein in the wheat and yield of flour*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	99	-0.2167 ± 0.0646
Minnesota spring wheats	202	$.1789 \pm .0459$
Montana spring wheats	34	$.3751 \pm .0994$
Ohio winter wheats	29	$-.2062 \pm .1199$
Montana winter wheats	91	$.0884 \pm .0702$
Minnesota winter wheats	43	$.3238 \pm .0921$
Pure strains:		
North Dakota spring wheats	56	$-.0942 \pm .0893$
Minnesota spring wheats	48	$.1255 \pm .0958$
Wisconsin winter wheats	22	$.0601 \pm .1433$

For the nine groups of data six coefficients are positive in signs and three negative. The correlations for the Ohio winter wheats, Montana winter wheats, North Dakota spring wheats, pure strains, Minnesota spring wheats, pure strains, and Wisconsin pure strains of winter wheats are not significant, since the value of the respective coefficients is less than three times their probable error. The remaining four coefficients indicate only a very slight correlation. Of these three are positive in sign and one is negative. The highest correlation, that for the Montana

spring wheats, is based upon data from only 34 samples. From the evidence furnished by these data it would seem that so far as flour yield is concerned, and within the above range of protein content, it matters little whether the miller buys wheats of high or low protein content. This is in conformation of the results obtained by Thomas (14) who states that—

> normal, plump, dry, and sound wheat of all classes yields approximately the same percentage of flour.

PROTEIN CONTENT IN WHEAT AND LOAF VOLUME

From a commercial point of view the value of a bread wheat is determined principally by the size of loaf baked from a unit of flour of that wheat. The size of bread loaf is generally measured by the volume, expressed in cubic centimeters, of the loaf baked from a unit of flour, usually 340 gm. The volume of bread loaf is at present the only reliable index of flour strength. It is, therefore, not surprising to find that various chemical characters have been studied in an effort to establish a relationship between them and all the important factor of flour strength.

The following data are intended to express the relationship, in the light of the available material, between the volume of bread loaf and the protein in the wheat, protein in the flour, dry gluten, wet gluten gliadin, and water absorption.

TABLE IX.—*Correlation between protein in the wheat and loaf volume from Ohio winter wheats of commercial varieties*

Protein in wheat (per cent).	Loaf volume (cc.)								
	1,600 to 1,700	1,700	1,800	1,900	2,000	2,100	2,200	2,300 to 2,400	Total.
8.00 to 8.50	.....	.....	.....	.....	.....	.....	.....	.....	.....
8.50	1	.....	3	1	.....	1	.....	.....	6
9.00	1	.....	3	4	2	.....	.....	.....	10
9.50	.....	2	3	1	2	.....	.....	.....	8
10.00	.....	.....	2	3	1	.....	.....	.....	6
10.50	1	1	2	1	3	1	1	.....	10
11.00	.....	.....	3	5	6	2	.....	.....	16
11.50	.....	.....	2	4	7	2	2	.....	17
12.00	.....	.....	.....	3	3	1	.....	.....	7
12.50	1	2	.....	.....	2	3	.....	.....	8
13.00	.....	.....	1	.....	.....	2	.....	.....	3
13.50	.....	.....	.....	.....	1	1	2	.....	4
14.00	.....	.....	.....	.....	.....	1	.....	.....	1
14.50	.....	.....	.....	.....	.....	1	.....	.....	1
15.00	.....	.....	.....	.....	.....	.....	1	.....	1
15.50	.....	.....	.....	.....	.....	.....	.....	1	1
16.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
16.50	.....	.....	.....	.....	.....	.....	.....	.....	.....
17.00	.....	.....	.....	.....	.....	.....	.....	1	1
17.50 to 18.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total...	4	5	19	22	27	15	6	2	100

$r = 0.5394 \pm 0.0479$.

TABLE X.—*Correlation coefficients for protein in wheat and loaf volume*

Kind of wheat.	Number of samples.	Coefficients of correlation.
Commercial varieties:		
North Dakota spring wheats	128	−0.1172±0.0588
Minnesota spring wheats	202	.1827± .0459
Montana spring wheats	34	.3555± .1011
Colorado spring wheats	48	.4908± .0739
Montana winter wheats	91	.3638± .0613
Ohio winter wheats	100	.5394± .0479
Minnesota winter wheats	43	.5874± .0674
Kansas winter wheats	42	.7547± .0448
Pure strains:		
Minnesota spring wheats	48	.4621± .0766
Wisconsin spring wheats	22	.5123± .1061
Maine spring wheats	31	.5194± .0885
Ohio winter wheats	25	.5548± .0934

The data in Tables IX and X, with the exception of those for North Dakota spring wheats and Minnesota commercial varieties of spring wheats, indicate a high, positive correlation. The coefficients of correlation range from -0.1172 ± 0.0588 for the North Dakota wheats to 0.7547 ± 0.0448 for the Kansas wheats. The negative correlation for the North Dakota wheats is not significant, since the value of the coefficient is less than three times its probable error. The coefficient for the Minnesota commercial varieties of spring wheats indicates only a slight, positive correlation. This is rather of interest since both States are centers of production of the hard red spring wheat. In this connection the observations of Thomas (*14*) may be cited which seem to throw some light on this point. Thomas found that high crude-protein content as a rule is accompanied by high strength but that the relation between these two factors varies with different classes of wheat, and extremely high crude-protein—over 15 per cent—is sometimes accompanied by a decrease in baking strength. The writer had occasion to observe several similar instances in pure lines selected from Preston wheats. Thomas further found that a wider variation in volume is noticeable with all classes of wheat for the lower percentages of protein than for the higher percentages, many samples with very high protein content being lower in strength than those having a medium content. In the light of these observations one may assume that the large number of samples with very high protein entering into the material from which the North Dakota and Minnesota data have been secured may have counteracted and neutralized the influence of the samples having a medium protein content. It should be noted, however, that the coefficients for the Minnesota pure strains of spring wheat indicate a rather high positive correlation.

PROTEIN IN FLOUR AND LOAF VOLUME

The high correlation between the protein content in the wheat and protein content in flour points to a correlation between the protein content in flour and loaf volume which will closely agree with that between the protein content in wheat and loaf volume.

TABLE XI.—*Correlation between protein in flour and loaf volume from Ohio winter wheats of commercial varieties*

Protein in flour (per cent)	VOLUME LOAF (cc.)								
	1,600 to 1,700	1,700	1,800	1,900	2,000	2,100	2,200	2,300 to 2,400	Total.
6.00 to 6.50	.....	.....	.....	.....	.....	.....	.....	.....	.....
6.50	1	.....	3	1	2	.....	.....	.....	7
7.00	1	.....	4	3	1	1	.....	.....	10
7.50	.....	1	3	2	2	.....	.....	.....	8
8.00	.....	.....	.....	3	1	1	1	.....	6
8.50	1	1	3	1	3	.....	1	.....	10
9.00	.....	1	1	3	3	2	.....	.....	10
9.50	.....	.....	2	4	.....	3	1	.....	10
10.00	.....	1	1	2	11	.....	.....	.....	15
10.50	.....	1	2	.....	1	3	.....	.....	7
11.00	.....	.....	.....	2	2	3	1	.....	8
11.50	1	.....	.....	.....	1	.....	.....	.....	2
12.00	.....	.....	.....	.....	.....	.....	1	.....	1
12.50	.....	.....	.....	.....	.....	1	.....	.....	1
13.00	.....	.....	.....	.....	.....	1	.....	.....	1
13.50	.....	.....	.....	.....	.....	.....	1	1	2
14.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
14.50	.....	.....	.....	.....	.....	.....	.....	.....	.....
15.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
15.50	.....	.....	.....	.....	.....	.....	.....	1	1
16.00 to 16.50	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	4	5	19	21	27	15	6	2	99

$r = 0.4709 \pm 0.0528$.

TABLE XII.—*Correlation coefficients for protein in flour and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation
Commercial varieties:		
North Dakota spring wheats	119	-0.0987 ± 0.0612
Minnesota spring wheats	203	$.2586 \pm .0442$
Montana spring wheats	34	$.3448 \pm .1019$
Colorado spring wheats	48	$.6130 \pm .0608$
Montana winter wheats	91	$.3620 \pm .0614$
Ohio winter wheats	99	$.4709 \pm .0528$
Minnesota winter wheats	43	$.6496 \pm .0594$
Kansas winter wheats	43	$.7956 \pm .0377$
Pure strains:		
North Dakota spring wheats	28	$.3018 \pm .1158$
Minnesota spring wheats	48	$.5469 \pm .0689$
Ontario spring wheats	16	$.5752 \pm .1129$
Wisconsin winter wheats	25	$.3990 \pm .1134$
Ohio winter wheats	25	$.5560 \pm .0932$

From these data, with one exception, the positive and generally high correlation between the loaf volume and protein in flour is evident. The correlation for the North Dakota spring wheats, commercial varieties, is again negative and without significance. An inspection of Tables X and XII further shows that the relative rank of values of the correlation coefficients is identical for both pairs of characters considered. A comparison of the absolute values of correlation coefficients for the two pairs

of variables reveals the fact, with few exceptions, the degree of correlation between loaf volume and protein in flour is higher than that between loaf volume and protein in wheat. The Ohio and Montana winter wheats constitute an exception to the general condition as indicated by the foregoing data. For the Ohio winter wheats, commercial varieties, the correlation between loaf volume and protein in flour is lower than that between loaf volume and protein in wheat (Tables IX and XI), while for the Montana winter wheats and Ohio winter wheats, pure strains, the degree of correlation between these two pairs of variables is practically identical.

DRY GLUTEN AND LOAF VOLUME

It is regretted that the data for this important pair of variables, both as to the number of wheat groups and number of samples within the groups, should be so small.

TABLE XIII.—*Correlation between dry gluten and loaf volume from Ohio winter wheats of commercial varieties*

Dry gluten (per cent).	Loaf volume (cc.).								Total.
	1,500 to 1,600	1,600	1,700	1,800	1,900	2,000	2,100	2,200 to 2,300	
6.50 to 7.00									
7.00				4	1				5
7.50	1	1		1	1	2			6
8.00		1	1	3	1	2			8
8.50		1	2	2	3	1			9
9.00				3	3	5			11
9.50				3	4	1	2		10
10.00				1	2	4			7
10.50					1	3		1	5
11.00									
11.50									
12.00						1			1
12.50						1			1
13.00 to 13.50									
Total	1	3	3	17	16	20	2	1	63

$r = 0.4770 \pm 0.0656$.

TABLE XIV.—*Correlation coefficients for dry gluten and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	37	0.0391 ± 0.1107
Colorado spring wheats	48	.7412 ± .0439
Ohio winter wheats	63	.4770 ± .0656
Pure strains:		
Maine spring wheats	31	.5707 ± .0806
Ontario spring wheats	16	.0078 ± .1663

The data in Tables XIII and XIV indicate, again with one exception, positive high correlations. The coefficients of correlation range from 0.0391 ± 0.1107 for the North Dakota spring wheats to 0.7412 ± 0.0439

for the Colorado spring wheats. The degree of correlation between dry gluten content and loaf volume for the Ohio winter wheats is practically identical with that between protein in flour and loaf volume for the same data, while with the other wheats the data indicate a higher correlation between gluten and loaf volume than that between protein in flour and loaf volume. The correlation for the North Dakota spring wheats is not significant.

WET GLUTEN AND LOAF VOLUME

What has been said about the paucity of data for the characters dry gluten and loaf volume holds also for the data on wet gluten and loaf volume.

TABLE XV.—*Correlation between wet gluten and loaf volume from Colorado spring wheats of commercial varieties*

Wet gluten (per cent).	Loaf volume (cc.)								Total.
	1,000 to 1,100	1,100	1,200	1,300	1,400	1,500	1,600	1,700 to 1,800	
21.00 to 23.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
23.00	.....	.....	.....	1	1	.....	.....	.....	2
25.00	.....	.....	.....	4	2	1	.....	.....	7
27.00	.....	.....	1	.....	.....	1	.....	.....	2
29.00	.....	.....	.....	.....	3	1	.....	.....	4
31.00	.....	2	.....	1	5	4	.....	.....	12
33.00	.....	.....	.....	.....	3	3	1	.....	7
35.00	1	.....	.....	.....	.....	3	1	.....	5
37.00	.....	.....	.....	.....	1	1	.....	.....	2
39.00	.....	.....	.....	.....	.....	1	1	.....	2
41.00	.....	.....	.....	.....	.....	1	.....	.....	1
43.00	.....	.....	.....	.....	.....	.....	1	.....	1
45.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
47.00	.....	.....	.....	.....	.....	.....	1	1	2
49.00	.....	.....	.....	.....	.....	.....	.....	1	1
51.00 to 53.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	1	2	1	6	15	16	5	2	48

$r = 0.5025 \pm 0.0728$.

TABLE XVI.—*Correlation coefficients for wet gluten and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	37	0.2600±0.1034
Colorado spring wheats	48	.5025± .0728
Ohio winter wheats	29	.5425± .0883
Pure strains:		
Maine spring wheats	31	.4573± .0958
Ontario spring wheats	16	.6062±. 1067

The coefficients for the North Dakota spring wheats and Ohio winter wheats show a higher correlation between the characters here considered than that between dry gluten and loaf volume. The correlation for the North Dakota wheats, however, is not significant. The correlation

between wet gluten and loaf volume for the Colorado and Maine wheats is lower than that between dry gluten and loaf volume, while the degree of relationship of the two pairs of variables is almost identical for the Ontario wheats.

WATER ABSORPTION AND LOAF VOLUME

Strong flours are regarded as being possessed of a higher capacity for water absorption than weak flours. From this a positive correlation between water absorption and loaf volume is to be expected.

TABLE XVII.—*Correlation between water absorption and loaf volume from Minnesota spring wheats of commercial varieties*

Water absorption (per cent).	Loaf volume (cc.).								Total.
	2,000 to 2,100	2,100	2,200	2,300	2,400	2,500	2,600	2,700 to 2,800	
47 to 48	.....	.....	.....	.....	.....	.....	.....	.....	.....
48	2	.....	.....	.....	.....	.....	.....	.....	2
49	.....	.....	.....	.....	1	.....	.....	.....	1
50	1	4	1	.....	2	.....	.....	.....	8
51	.....	.....	.....	.....	2	.....	.....	.....	2
52	.....	1	2	.....	1	2	.....	.....	6
53	.....	.....	.....	.....	2	2	2	.....	6
54	.....	.....	2	.....	1	4	2	1	10
55	.....	1	2	1	.....	.....	2	.....	6
56	.....	1	1	2	1	6	3	.....	14
57	.....	2	.....	4	7	5	5	.....	23
58	.....	.....	2	1	3	7	4	.....	17
59	.....	.....	1	1	8	20	3	.....	33
60	.....	.....	1	1	9	12	.....	2	25
61	1	.....	.....	1	3	12	2	.....	19
62	.....	.....	.....	.....	3	8	1	.....	12
63	.....	.....	.....	1	5	4	2	.....	12
64	.....	.....	.....	.....	1	1	.....	.....	2
65	.....	.....	.....	.....	1	1	1	.....	3
66 to 67	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	4	9	12	12	50	84	27	3	201

$r = 0.3760 \pm 0.0408$.

TABLE XVIII.—*Correlation coefficients for water absorption and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation
Commercial varieties:		
North Dakota spring wheats	119	0.2090 ± 0.0501
Montana spring wheats	34	.2546 ± .1082
Minnesota spring wheats	201	.3760 ± .0408
Montana winter wheats	91	.0348 ± .0706
Minnesota winter wheats	43	.4765 ± .0795
Ohio winter wheats	86	.5201 ± .0524
Kansas winter wheats	42	.0601 ± .0587
Pure strains:		
Minnesota spring wheats	48	.0100 ± .0973
North Dakota spring wheats	27	.4110 ± .1078
Ohio winter wheats	31	.1498 ± .1184

Of the 10 coefficients of correlation given in Tables XVII and XVIII, 3—those for Montana winter wheats, Minnesota spring wheats, pure strains, and Ohio winter wheats, pure strains—indicate no significant correlation, since their value is considerably less than three times their probable error. The correlation for the winter wheats are, with two exceptions, higher than for the spring wheats.

FLOUR YIELD AND LOAF VOLUME

The coefficients of correlation for this pair of variables do not indicate a consistent trend. Of the eight coefficients, four are positive in sign and four negative. Five coefficients indicate virtually no correlation, as their value is less than three times their probable error. Of the remaining three coefficients, two indicate only a slight positive correlation for the Minnesota, winter, and spring wheats, and one the highest but negative correlation, -0.3920 ± 0.0763 for the North Dakota spring wheats, pure lines. In considering these data the influence of the condition of wheat upon the flour yield should be borne in mind, the shrunken wheat often yielding less flour and a larger volume of bread loaf than very plump, sound wheat.

TABLE XIX.—*Correlation between yield of flour and loaf volume from Minnesota spring wheats of commercial varieties*

Yield of flour (per cent).	Loaf volume (cc.).								Total.
	2,000 to 2,100	2,100	2,200	2,300	2,400	2,500	2,600	2,700 to 2,800	
64–65	.....	.....	.....	.....	.....	.....	.....	.....	.....
65	.....	.....	1	.....	2	.....	.....	.....	3
66	.....	.....	1	2	.....	.....	.....	.....	3
67	.....	.....	.....	1	2	5	.....	.....	8
68	.....	2	.....	.....	1	2	.....	.....	5
69	1	.....	2	3	2	11	5	.....	24
70	.....	2	5	1	14	12	4	.....	38
71	.....	2	.....	2	7	16	5	.....	32
72	.....	1	1	2	4	18	1	.....	27
73	3	2	1	1	13	15	10	2	47
74	.....	.....	.....	.....	2	4	1	1	8
75	.....	.....	1	.....	3	.....	1	.....	5
76	.....	.....	.....	.....	.....	2	.....	.....	2
77–78	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	4	9	12	12	50	85	27	3	202

$r = 0.1524 \pm 0.0464$.

TABLE XX.—*Correlation coefficients for yield of flour and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	99	−0.0900±0.0672
Montana spring wheats	34	.1425± .1133
Minnesota spring wheats	202	.1524± .0464
Montana winter wheats	91	− .0530± .0705
Ohio winter wheats	29	− .1440± .1227
Minnesota winter wheats	43	.3392± .0910
Pure strains:		
North Dakota spring wheats	56	− .3920± .0763
Minnesota spring wheats	48	.1283± .0958

GLIADIN AND LOAF VOLUME

As already stated, the gliadin content has been suggested as a factor determining strength in wheat flour. In Table XXI are given data indicating the relationship between gliadin and strength as measured by loaf volume. The correlation for the North Dakota wheats, commercial varieties, is again negative, that for the Colorado is positive, but both are hardly significant as their value is less than three times their probable error. The remaining correlations are positive and some of them very high, ranging from 0.3815±0.0960 for the Kansas wheats to 0.7455± 0.0598 for the North Dakota spring wheats, pure strains.

TABLE XXI.—*Correlation coefficients for gliadin and loaf volume*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	113	−0.1718±0.0616
Colorado spring wheats	48	.0308± .0973
Kansas winter wheats	36	.3815± .0960
Pure strains:		
Ontario spring wheats	16	.6304± .1017
North Dakota spring wheats	25	.7455± .0598

DRY GLUTEN AND GLIADIN

Gliadin is one of the constituents of gluten, and a close association between these two values is to be expected. The data in Tables XXII and XXIII are rather meager and indicate a positive correlation, the coefficients ranging from 0.3941±0.1058 for the Ohio wheats, which is rather low, to 0.9352±0.0212 for the Ontario wheats.

TABLE XXII.—*Correlation between dry gluten and gliadin content of North Dakota spring wheats of commercial varieties*

Dry gluten (per cent).	Gliadin (per cent).											Total.
	4.00 to 4.50	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00	8.50	9.00 to 9.50	
9.00 to 9.50												
9.50							1					1
10.00												
10.50												
11.00				1								1
11.50												
12.00					1	1						2
12.50							2					2
13.00				1	1	2						4
13.50						1	1	2				4
14.00												
14.50	1					1		2				4
15.00								3				3
15.50							1		3	1		5
16.00							1			2		3
16.50											1	1
17.00										1	1	2
17.50							1					1
18.00								1				1
18.50											1	1
19.00									1			1
19.50 to 20.00												
Total	1			2	2	5	7	8	4	4	3	36

$r = 0.6107 \pm .0704$.

TABLE XXIII.—*Correlation coefficients for dry gluten and gliadin content*

Kind of wheat	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	36	0.6107 ± 0.0704
Ohio winter wheats	29	.3941 ± .1058
Pure strains:		
Ontario spring wheats	16	.9352 ± .0212

DRY GLUTEN AND WATER ABSORPTION

Since high gluten content and water absorption are generally associated with high baking strength as measured by loaf volume (Tables XIII, XIV, XVII, and XVIII) one might expect some relationship between dry gluten content and water absorption.

TABLE XXIV.—*Correlation between dry gluten content and water absorption in Ohio winter wheats of commercial varieties*

Dry gluten (per cent).	Water absorption (per cent).													Total.
	56 to 57	57	58	59	60	61	62	63	64	65	66	67	68 to 69	
6.50 to 7.00	....	....	....	....	....	....	....	....	....	....	....	....	....	....
7.00	....	1	....	....	1	2	1	....	....	....	....	....	....	5
7.50	....	1	1	1	1	....	1	1	....	....	....	....	....	6
8.00	....	1	3	....	1	2	1	....	....	....	....	....	....	8
8.50	2	2	1	....	2	....	....	....	1	....	....	....	1	9
9.00	....	1	3	1	3	2	1	....	....	....	....	....	....	11
9.50	....	1	....	1	4	2	....	....	1	1	....	....	....	10
10.00	....	....	....	....	2	5	....	....	....	....	....	....	....	7
10.50	....	1	....	....	....	1	....	2	....	....	....	....	1	5
11.00	....	....	....	....	....	....	....	....	....	....	....	....	....	....
11.50	....	....	....	....	....	....	....	....	....	....	....	....	....	....
12.00	....	....	....	....	....	....	1	....	....	....	....	....	....	1
12.50	....	....	....	....	1	....	....	....	....	....	....	....	....	1
13.00 to 13.50	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Total	2	8	8	3	15	14	5	3	2	1	....	....	2	63

$r = 0.2211 \pm 0.0808$.

TABLE XXV.—*Correlation coefficients for dry gluten content and water absorption*

Kind of wheat.	Number of samples.	Coefficient of correlation.
Commercial varieties:		
North Dakota spring wheats	37	0.1406±0.1087
Ohio winter wheats	63	.2211± .0808
Pure strains:		
Ohio winter wheats	19	−.1319± .1520

The data in Tables XXIV and XXV do not bear out this expectation, since they indicate hardly any significant correlation. An interpretation of these data would lead to the conclusion that flours with high gluten content behave indifferently in regard to water absorptive capacity. It is only fair, however, to note the small number of samples from which these correlation coefficients have been computed. Caution should be exercised in interpreting the value of the correlation coefficients as indicated by the probable errors in cases where the number of samples is small.

QUALITY OF GLUTEN AND LOAF VOLUME

Practical experience in the bakehouse shows that strength in wheat flour is best reflected by the quality of its gluten. The measuring of the intensity of the association between these two characters can not be effected by computing the coefficient of correlation, since one of these variables, quality, can not be numerically defined but is only estimated in descriptive terms. Accordingly, as a measure of the degree of association between gluten quality and loaf volume the correlation ratio,

η, has been computed. Eight degrees of gluten quality have here been considered ranging from very soft and sticky, dead, nonelastic gluten to very good, strong, very elastic. In Table XXVI are given the correlation ratios for three groups of wheat.

TABLE XXVI.—*Correlation ratio, η, for quality of gluten and loaf volume*

Kind of wheat.	Number of samples.	Correlation ratio.
Pure strains:		
Maine spring wheats	31	0.7418±0.0544
Ontario spring wheats	16	[a].9478± .0171
Wisconsin winter wheats	16	.7456± .0748

[a] Because of its small number of observations the regression for this table is not linear. The value of the coefficient is too high. The regression lines for the other two tables appear to be linear.

From these data the very high and consistent relationship between gluten quality and loaf volume will be noted. Notwithstanding the paucity of data and the fact that the constant η generally has a somewhat greater value for the same data than the coefficient of correlation, it is safe to conclude that loaf volume is more closely associated with gluten quality than with any other character considered. While it is no doubt true that the character quality of gluten is determined by estimation and hence may be subject to variation due to personal equation, yet the determinations of gluten quality made according to a definite standard in a given laboratory may be regarded as fairly constant.

DRY GLUTEN CONTENT AND QUALITY OF GLUTEN

In considering the results of a chemical analysis of flours from pure lines of wheat grown in Aroostook, the writer (18, p. 37) noticed that while high gluten content in flours from pure wheat strains selected from a given commercial variety was not generally associated with good quality of gluten, yet the flours from pure strains of the same variety showing a low gluten content were generally also low in gluten quality. The degree of relationship between gluten content and gluten quality as measured by the constant η is given in the data in Table XXVII.

TABLE XXVII.—*Correlation ratio, η, for dry gluten content and quality of gluten*

Kind of wheat.	Number of samples.	Correlation ratio.
Pure strains:		
Maine spring wheats	31	0.5994±0.0777
Ontario spring wheats	16	[a].6565± .0958
Wisconsin winter wheats	16	.5155± .1237

[a] As in Table XXIV, the regression lines for the Ontario spring wheats are not linear, the value of η comes chiefly from the zigzag nature of the regression lines and not from any true relationship between the variables. The lines for the Maine and Wisconsin wheats appear to be linear.

The number of flour samples for which these two variables have been determined is small, but the data generally indicate a fairly high correlation between quantity and quality of gluten. This relationship accounts for the frequently recorded observation that glutenous wheats are strong

and furnish a larger loaf volume from a unit of flour than wheats with a low gluten content. The relationship is by no means perfect, probably because of the fact that a number of wheats with high gluten content fail to show good quality of gluten. In this connection it should be noted that the relationship between these two variables is to be studied for pure strains belonging to a given variety or group of wheat and considered within that group since there is some evidence indicating that certain wheat groups carry a larger number of varieties or strains of very high gluten content of poor quality than others. The durum and Preston wheats may be cited as illustrating this point.

DISCUSSION OF RESULTS

The relations considered in the preceding section will now briefly be considered from the standpoint of the plant breeder who desires to make use of any correlation between a wheat character determinable at an early stage of the pure line selection work and the baking strength of flour.

Tables I to VI show data expressing the relationship between the crude protein in the wheat and its constituents, protein in flour, gluten, and gliadin. The correlations are very high and consistent, as would be expected. The correlation between crude protein content in the wheat and that in flour is so high that, for practical purposes, the value for the protein content in the flour may be substituted for that in the wheat. This would eliminate the necessity of securing a sample of flour for analysis, which requires a larger amount of grain and an experimental mill to grind it, whereas for the determination of crude protein in the grain the yield of a single plant is sufficient.

Of great interest are the correlations between loaf volume and the other chemical characters. The data given in Tables IX to XII indicate that the relation between loaf volume and crude protein in the wheat is paralleled by that between loaf volume and protein in the flour, the difference being one of degree as the relationship for the latter pair of characters is of greater intensity than that for the former. Since the correlation between loaf volume and protein in the flour parallels that between loaf volume and crude protein in the wheat, it would follow that, again for practical purposes, it may be sufficient to consider the crude protein in the wheat alone in relationship to strength of flour.

Considering the gluten content and loaf volume, the data in Tables XIII and XIV, which are rather scant, indicate a very high, positive correlation between these two variables. Gliadin, which is one of the constituents of gluten and closely correlated with it (Tables XII and XXIII), generally bears a close relation to loaf volume (Table XXI).

Finally, considering the quality of gluten and loaf volume, it will be seen from Tables XXVI and XXVII that loaf volume is more closely correlated with gluten quality than with any other character under consideration.

From this brief consideration of the more important correlations it should be clear that as far as strength of flour is concerned gluten content and gluten quality show the highest degree of association with loaf volume. If these correlations are accepted as a working basis, the problem of finding a practical index of strength of flour is reduced to the task of determining the gluten content and gluten quality. In order to determine these two values a certain amount of flour is required. This is not avail-

able until after the propagation of the pure strains of wheat has reached a certain stage. In order to judge the gluten quality, when only a very small sample of grain is available, say the yield of a single plant, or even of a spike, use may be made of a rather crude but fairly accurate method, the chewing test. The chewing test gives also some approximate idea as to gluten content. The gluten content well reflects, in fact, parallels the crude protein content in the wheat. Furthermore, both the gluten content and crude protein content are highly correlated with loaf volume. From this it would follow that the crude protein content in the wheat may, for practical purposes, be substituted for gluten content. Thus the crude protein content in the wheat and the chewing test may be accepted as a practical working index of strength in wheat in the early stages of the selection work when the plant breeder is confronted with the task of deciding which of the numerous strains to retain and which to discard. The information afforded by this index may further be supplemented by examining the grain for color and hardness.

The determination of the crude protein content in the grain of a given strain gains in importance and value in view of the accumulating evidence which indicates that while the absolute amount of protein in wheat may be influenced by environment, yet the protein content of wheat is a varietal characteristic, and moreover, that there is a tendency for the different varieties and strains to retain their relative rank with respect to protein content from one year to the next (*18*).

SUMMARY

The present paper is the result of a study of the relationship of the different characters of wheat based upon published data obtained from analysis of a number of American wheats.

Subject to the limitations of the material which have been pointed out in this paper, the following conclusions may be drawn from the data herein presented:

Crude protein content in the wheat is very closely and consistently correlated with protein in flour, dry gluten, and gliadin.

There appears to be practically no relation between crude protein content in the wheat and flour yield.

There is, with some notable exceptions, a high positive correlation between the crude protein in the wheat and strength of flour as determined by the loaf volume.

There is generally even a higher, positive correlation between protein in the flour and loaf volume.

The gluten content of the flour is very closely correlated with loaf volume. The intensity of association between these two variables appears to be greater than that for protein in flour and loaf volume.

There is generally a high, positive correlation between wet gluten content and loaf volume.

There is, with some exceptions, a positive, fairly high correlation between water absorption of the dough and loaf volume.

In normal, sound wheat there is apparently no significant correlation between flour yield and loaf volume.

Excepting the data for a few wheat groups, there is a positive and, for some wheat groups, a very high correlation between gliadin content and loaf volume.

Dry gluten content is generally highly correlated with gliadin content.

From the scant data for dry gluten content and water absorption it would seem that there is no significant correlation between these two variables, which is contrary to expectation.

Loaf volume is more closely associated with gluten quality as indicated by the correlation ratio than with any other character considered.

There appears to be a fairly high correlation between the content and quality of gluten.

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APLASTOMORPHA VANDINEI TUCKER, AN IMPORTANT PARASITE OF SITOPHILUS ORYZA L.[1]

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INTRODUCTION

The most effective insect enemy of the rice weevil, *Sitophilus oryza* L., in the United States is the chalcid *Aplastomorpha vandinei* Tucker.

While engaged in studying the biology of the rice weevil at Orlando, Fla., during the years 1920 and 1921, the writer made the following observations on the life history and habits of this parasite.

ORIGIN AND DISTRIBUTION

Although originally described as from Texas, the native home of this chalcid is unknown. It is now cosmopolitan in its distribution, probably occurring wherever corn is used.

HISTORY AND SYNONYMY

The earliest definite reference to this species in literature appeared in 1899, when Chittenden (*2*)[2] recorded it as parasitic on *Bruchus quadrimaculatus* Fab. under the name of *Apalastomorpha prattii* Ashm. MSS.

No description of this species was published, however, until 1910, when Tucker (*10*) described it under the name of *Meraporus vandinei* from Plano, Tex.

In 1913 the same species was described by Crawford (*3*) as *Aplastomorpha pratti*, the manuscript name previously given by Ashmead to individuals of this species bred in Washington, D. C., from *Bruchus quadrimaculatus* Fab. Crawford's description was based on specimens collected in Dallas, Tex., by W. D. Hunter.

Later in the same year the species was again described, this time by Girault (*5*), under the name *Neocatolaccus australiensis*, from material reared in Australia.

In 1915 Girault (*6*) placed his species *australiensis* in the genus Aplastomorpha Crawford and in 1917 (*7*) reduced *Aplastomorpha pratti* Craw., *A. australiensis* Gir., and *Meraporus vandinei* Tucker to synonymy with *Neocatolaccus vandinei* Tucker.

Finally Gahan (*4*) in 1920 recognized *Aplastomorpha vandinei* Tucker as the correct name of the species.

ECONOMIC IMPORTANCE

Aplastomorpha vandinei is of chief economic importance as a parasite of the grain weevils *Sitophilus oryza* and *S. granarius* L. It has also been reported as parasitic on several other insects of economic importance—that is, on the 4-spotted bean weevil, *Bruchus quadrimaculatus*, by Chittenden (*2*), on the cigarette beetle, *Lasioderma serricorne* Fab., by Rumner (*9*) and Bodkin (*1*), on *Pachymerus* sp. by Girault (*8*), and on the "grain

[1] Accepted for publication May 13, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 556.

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moth" and miscellaneous forest galls by Girault (5). The writer has bred it from *Sitophilus oryza*, *S. granarius*, and also from the broad-nosed grain weevil, *Caulophilus latinasus* Say.

DESCRIPTION OF THE SPECIES

The female (Pl. 1, A) is normally about 2 mm. in length, of characteristic chalcid form, dark metallic green in color, with yellow legs. The male (Pl. 1, G) is somewhat smaller and differs from the female in having the abdomen brown, with a large yellowish white spot. Both sexes vary considerably in size.

TECHNICAL DESCRIPTION (*10*)

Female: Head and thorax dark greenish, clothed with thin and fine silvery pubescence, a thick linear patch on each side of the metathorax; abdomen smooth, shining greenish, thinly pubescent on apical segments, venter deeply keeled; head transverse, somewhat wider than thorax, finely reticulated rather than punctured on occiput, front and cheeks with convergent striæ at oral margin of middle of face; front above insertion of antennæ hollowed for reception of scapes; mouth-parts ferruginous, each mandible with four denticles; antennæ about as long as thorax, scape dull reddish; flagellum dull reddish beneath, darker above, with fine silvery pubescence; pedicel about twice the length of the three ring joints together, but scarcely longer than the first funicle joint, which appears distinctly longer than wide, second and third funicle joints slightly longer than wide, fourth and fifth quadrate; club expanded at junction of first and second joints, the third forming a small conical tip. Anterior ocellus situated but little in advance of a median point between the posterior ones.

Thorax with fine shallow thimble-pitted punctures, contiguous and distinctly larger than on head; length of thorax scarcely exceeds the width, parapsidal furrows very faint only on anterior half of mesonotum. Metathorax very finely punctured, with a median longitudinal carina; metathoracic neck very short, smooth; lateral folds indicated by basal foveolæ only, spiracles very small, broadly oval; spiracular sulci very deep and distinct.

Tegulæ fulvous; wing-veins yellow, ciliate, stigmal vein shorter than marginal or postmarginal by about one-fourth the length, legs yellow, excepting the coxæ, femora in greater part between the base and apex, and last tarsal joint outwardly, which are dark brown or fuliginous. Tibiæ of middle and posterior legs little longer than femora or tarsi, which are about equal in length, but no noticeable difference in these respects with fore legs. Comparatively the fore legs are shorter than the others. [Length ranges from 1.25 mm. to 2 mm.]

Male: Antennæ with larger microscopical pits than female; the first and second ring joints very small and compressed, the third appears as the first joint of the funicle, but is smaller and shorter than the true funicular joints. Abdomen hardly as long, or at most not longer than thorax, almost flattened above, not deeply keeled ventrally, widest near apex, and with a dorsal fulvous area near base expanding to the lateral edges; otherwise agreeing with female. [Length ranges from 1 mm. to 1.5 mm.]

METHODS USED IN STUDY OF PARASITES

The immature stages of the rice weevil, *Sitophilus oryza*, which served as a host for the parasite in this study, are passed entirely concealed within the seeds of some of our common grains; hence of necessity the immature stages of the attacking parasite are also concealed from sight within the recesses of the grain that harbors the weevil. In order to observe the method of attack and the subsequent development of the parasite the following method was devised: Larvæ of the rice weevil were placed singly in small gelatin capsules, a small piece of absorbent cotton being packed in behind them to hold them in place. The capsules thus loaded were then placed in glass vials containing parasites.

The parasites in every instance accepted the loaded capsules in lieu of infested grain, located the weevil larvæ, pierced the shell of the capsules with their slender ovipositors, stung the larvæ into quiescence, and deposited their eggs.

Since the gelatin capsules were transparent, every phase of the attack and oviposition was clearly visible. After oviposition had taken place the capsules were removed and kept for observation. The eggs hatched and the young parasitic grubs developed to maturity in a perfectly normal manner, all changes being readily observed.

In addition to being able to observe the development of the parasites it was a simple matter to count the number of eggs deposited daily by individual parasites.

It was found that by pricking small holes through the shell of the capsules in the vicinity of the enclosed larvæ, the parasites would avail themselves of the holes to attack the larvæ and deposit eggs instead of going to the trouble of boring through the gelatin.

The parasites were fed on a sirup made from sugar and water.

LIFE HISTORY OF PARASITE

COPULATION

Copulation normally takes place shortly after emergence. Sex attraction is weak, the male showing no evidence of interest except when in close proximity to the female. In captivity the female more often seeks the male at first, moving around in the proximity of the male and stopping to preen her wings and body. After becoming conscious of her presence the male evinces signs of excitement, follows her about, and springs to her back. He then caresses antennæ with her for a brief period before copulating. In the act of copulation the female tilts her abdomen, and the abdomen of the male is curved down at one side of or directly over the end of the abdomen of the female while he clings to her wings and body. Copulation lasts for from 5 to 35 seconds.

OVIPOSITION

Oviposition usually begins on the first or second day after the emergence of the female, although it may begin the day of emergence. The stage of the host attacked is apparently of no great moment as long as it is large enough to permit the parasitic grub to develop to maturity. The fourth larval, prepupal, and pupal stages are all attacked, though the preference seems to lie with the larval stage.

The female parasite crawls around over the infested grain, exploring carefully the surface of the grain with the tips of her antennæ. Possibly the movements of the feeding weevil grub are detected by the sensitive tips of the antennæ; at any rate, the position of the grub is soon located. The ovipositor is brought into position with the tip of the abdomen, thrust through the shell of the grain, and plunged into the weevil grub. The ovipositor is apparently whipped around just under the skin until the grub is reduced to a paralyzed or quiescent state. The ovipositor is then withdrawn. It is again thrust through the shell of the grain and an egg is deposited on the exterior of the grub or in close proximity to it.

Only one egg is deposited at a time and usually but one egg is deposited with each larva or pupa attacked. With the parasites under observation it was not uncommon for two, three, four, or even more eggs to be deposited on one larva, but this was probably due in part to the effects of confinement and the limited supply of host material.

When several eggs are deposited on a single host grub it is rarely that more than one parasite reaches maturity; the writer has, however, observed a few instances where two parasites were reared on the same host.

OVIPOSITION PERIOD

The oviposition period extends over the greater part of the life of the female parasite. Commencing a day or two after emergence it lasts to within a week or two before death. The longest oviposition period recorded was 73 days, from December 4, 1920, to February 14, 1921, the shortest 39 days, from August 2, 1920, to September 9, 1920.

Eggs are laid at the rate of from 1 to 12 a day, depending upon the temperature. During the summer months the oviposition rate is high, but in winter the rate decreases to 1 or 2 a day. On the colder days oviposition ceases entirely.

NUMBER OF EGGS LAID

The greatest number of eggs laid by a single female was 283. These were laid in the months of August and September, 1920, over a period of 43 days. The lowest number recorded was 51. These were laid during the months of December, 1920, and January and February, 1921, over a period of 73 days. In general more eggs are laid by the summer generations of parasites than by those of winter.

Table I contains data concerning the oviposition period, the number of eggs laid, longevity, etc.

TABLE I.—*Data concerning oviposition and longevity of Aplastomorpha vandinei at Orlando, Fla., 1920–21*

No.	Date parasite emerged.	Date first egg was laid.	Length of preoviposition period.	Date last egg was laid.	Length of oviposition period.	Number of eggs laid.	Date of death.	Length of life.
			Days.		*Days.*			*Days.*
1	July 28	July 29	1	Sept. 9	43	283	Sept. 10	45
2	July 29	July 30	1	Sept. 21	54	258	Oct. 11	75
[1] 3	Aug. 11	Aug. 11	½	Sept. 29	50	157	Nov. 8	90
4	Aug. 17	Aug. 18	1	Oct. 24	68	236	Nov. 4	80
5	...do.....	...do.....	1	Oct. 27	71	239	Nov. 1	77
6	Aug. 20	Aug. 21	1	Oct. 30	71	279	Nov. 12	85
7	Oct. 15	Oct. 17	2	Dec. 22	67	93	Jan. 10	88
8	Nov. 1	Nov. 3	2	Jan. 12	71	52	Jan. 21	82
9	Nov. 21	Nov. 26	5	Feb. 5	72	79	Feb. 15	87
10	Dec. 2	Dec. 4	2	Feb. 14	73	51	...do.....	76

[1] Unfertilized female.

THE EGG

The egg (Pl. 1, F) is opaque shining white, the surface somewhat roughened with minute raised spots; it varies considerably in shape but is usually somewhat spindle-shaped, with one end thicker and more rounded than the other. Length 0.46 to 0.5 mm., width 0.14 to 0.16 mm.

INCUBATION PERIOD

The egg hatches in from 1½ to 2 days during the warm months of the year, but in winter the incubation period is lengthened to as many as 5 days.

The developing embryo may be seen near the center of the egg as a cloudy white oval body. As the embryo develops the contents of the egg draw away from the two ends of the tough chorion, which shrivels and wrinkles. In the process of hatching, the chorion is ruptured at the broad end of the egg and the young larva emerges.

LARVA STAGE

The newly emerged larva is about 0.5 mm. long and 0.20 mm. wide. It is a footless, fleshy grub, widest at the head and thorax and tapering caudad. It is transparent, smooth, and shining; body composed of 13 segments besides the head; head provided with a pair of slender chitinized mandibles (Pl. 1, B) 0.019 to 0.02 mm. in length.

If the egg is not attached to the host, the larva on hatching begins at once to seek for food, moving toward the host grub with an undulating movement of the body segments. Upon reaching its host the larva wanders about for a while over the grub but soon settles down to feed. It will feed on almost any part of the host.

The parasitic grub feeds rapidly and in summer becomes full grown in from 3 to 5 days. During cold weather the larval period is somewhat prolonged. Table II will give an idea of the variation in the length of this period at various times of the year.

TABLE II.—*Life-history data on Aplastomorpha vandinei*

No.	Date egg laid.	Date egg hatched.	Length of egg stage.	Date pupated.	Length of larval stage.	Date adult emerged.	Length of pupal stage.	Period from egg to adult.
			Days.		*Days.*		*Days.*	*Days.*
1	July 27	July 29	2	Aug. 3	5	Aug. 11	8	15
2	Aug. 3	Aug. 5	2	Aug. 10	5	Aug. 17	7	14
3	Aug. 5	Aug. 7	2	Aug. 12	5	Aug. 19	7	14
4	Aug. 5	Aug. 7	2	Aug. 11	4	Aug. 18	7	13
5	Aug. 6	Aug. 8	2	Aug. 12	4	Aug. 18	6	12
6	Aug. 13	Aug. 15	2	Aug. 18	3	Aug. 24	6	11
7	Aug. 13	Aug. 15	2	Aug. 18	3	Aug. 25	7	12
8	Aug. 14	Aug. 16	2	Aug. 20	4	Aug. 27	7	13
9	Aug. 15	Aug. 17	2	Aug. 21	4	Aug. 28	7	13
10	Aug. 22	Aug. 24	2	Aug. 27	3	Sept. 3	7	12
			[1] 2		[1] 4		[1] 7	[1] 13
11	Oct. 19	Oct. 21	2	Oct. 26	5	Nov. 9	14	21
12	Oct. 20	Oct. 23	3	Oct. 28	5	Nov. 10	13	21
13	Oct. 21	Oct. 23	2	Oct. 28	5	Nov. 11	14	21
14	Oct. 26	Oct. 28	2	Nov. 8	11	Nov. 23	15	28
15	Oct. 28	Oct. 30	2	Nov. 8	9	Nov. 27	19	30
16	Nov. 2	Nov. 4	2	Nov. 12	8	Dec. 7	25	35
17	Nov. 6	Nov. 8	2	Nov. 18	10	Dec. 12	24	36
18	Nov. 9	Nov. 11	2	Nov. 23	12	Dec. 28	35	49
19	Nov. 23	Nov. 25	2	Dec. 13	18	Jan. 8	26	46
20	Nov. 30	Dec. 5	5	Dec. 22	17	Jan. 19	28	50
			[2] 2.4		[2] 10		[2] 21.3	[2] 33.7
21	Dec. 2	Dec. 6	4	Dec. 23	17	Jan. 23	31	52
22	Dec. 4	Dec. 8	4	Dec. 23	15	Jan. 13	21	40
23	Dec. 5	Dec. 9	4	Dec. 27	18	Jan. 19	23	45
24	Dec. 7	Dec. 11	4	Dec. 27	16	Jan. 20	24	44
25	Dec. 8	Dec. 12	4	Dec. 28	16	Jan. 22	25	45
26	Jan. 3	Jan. 8	5	Jan. 21	13	Feb. 17	27	45
27	Jan. 5	Jan. 9	4	Jan. 27	18	Feb. 20	24	46
28	Jan. 13	Jan. 17	4	Feb. 3	17	Feb. 20	17	38
29	Jan. 14	Jan. 18	4	Feb. 5	18	Feb. 20	15	37
30	Jan. 19	Jan. 23	4	Feb. 10	18	Feb. 27	17	39
			[3] 4		[3] 16.6		[3] 22.4	[3] 43.1

[1] Summer average. [2] Fall average. [3] Winter average.

DESCRIPTION OF LARVA

The full-grown larva (Pl. 1, D) is of typical chalcid form; widest at the middle, it tapers both cephalad and caudad; smooth and shining in appearance, white in color but muddy-looking from the contents of the alimentary canal, which is greatly distended and appears nearly to fill the body cavity. The head is fairly prominent, bearing two tubercules and several small setæ, mouth-parts consisting mainly of two chitinized mandibles (Pl. 1, C) 0.053 to 0.057 mm. in length; mesothoracic, metathoracic, and first 7 abdominal segments bearing a pair of spiracles. The thoracic segments are provided with 4 setæ on each side. Abdominal segments 1 to 9, inclusive, have a subdorsal and a sublateral row of setæ on each side, 1 seta per segment in each row. The anal segment has a dorsal and ventral lobe, dorsal lobe has 4 setæ, and ventral lobe has 2 setæ. Length 2.7 to 3.0 mm., width 1.0 to 1.2 mm.

PREPUPAL STAGE

When full-grown the larva voids the entire contents of the alimentary tract. It becomes strikingly white in color and assumes a quiescent or prepupal stage. This stage normally lasts but a few hours, when the true pupal form is assumed.

PUPA

The pupa (Pl. 1, E), is at first perfectly white in color but soon turns to a pale yellowish brown, the eyes and ocelli turning reddish, and the mandibles brown. As the pupa gets older the eyes turn a darker red, and the thorax becomes dark in color followed by the head. Finally the abdomen of the male becomes banded with black and that of the female entirely black. The sexes may be readily distinguished in this stage by the difference in size, the females being considerably larger than the males. Male: Length 1.45 to 2.0 mm., width 0.61 to 0.79 mm. Female: Length 2.2 to 2.4 mm., width 0.90 to 1.0 mm.

The pupal stage lasts for a period of from 6 to 7 days. The males usually emerge in 6 days, while the females take 7. In winter the pupal stage is considerably prolonged, sometimes as long as 30 days.

HABITS OF ADULTS

In order to test the effect of light and darkness on the activity of the parasites, experiments were conducted in which corn infested with *Sitophilus oryza* was placed in containers from which the light was excluded. It was so arranged that the parasites could leave the lighted tubes in which they were placed and enter the dark containers at will.

It was found that the parasites would enter the darkened containers in search of their prey without any hesitation, so that we may assume that their beneficial work will be carried on equally as well whether the corn is stored in the light or the dark.

Female parasites were frequently observed to feed on the body juices of the weevil grubs. After jabbing the grubs with their ovipositors they lap up the exuding juices.

PARTHENOGENESIS

Unfertilized females kept under observation laid fertile eggs which invariably developed into males. These females lived as long as the average fertilized ones or longer but laid only about half as many eggs.

NUMBER OF MALES AND FEMALES

Males are apparently more abundant than females. Of reared specimens 60 per cent were males.

LONGEVITY

The average female parasite in captivity lived for about 82 days. This period is considerably longer than the life of the male, which averaged 47 days.

One female parasite that was not allowed to deposit eggs but was fed on sugar and water lived from August 19 to December 4, 1920, a period of 107 days.

Parasites confined without food and without weevil grubs lived for only a few days; males so confined died by the end of the sixth day and females by the ninth.

COMPARISON OF LIFE-HISTORY STATISTICS WITH THOSE OF SITOPHILUS ORYZA

With an average length of life about half that of the rice weevil, this parasite lays fully as many eggs per day and completes its life cycle in a little less than half the time taken by the rice weevil.

A smaller percentage of females is produced by the parasite, however, and several eggs of the parasite may be wasted on one host grub, so that the rate of multiplication is below that of the rice weevil and a complete control is not obtained.

Table III gives a comparison of the life-history records of the parasite and the rice weevil.

TABLE III.—*Life-history statistics of Aplastomorpha vandinei and Sitophilus oryza compared*[1]

Insect.	Average length of life.	Average length of oviposition period.	Average number of eggs laid.	Average number of eggs laid daily.	Males emerging.	Females emerging.	Length of life cycle.
	Days.	*Days.*			*Per cent.*	*Per cent.*	*Days.*
S. oryza	111	93.9	380	4	48	52	35
A. vandinei	72	61	259	4	60	40	14

[1] Summer records.

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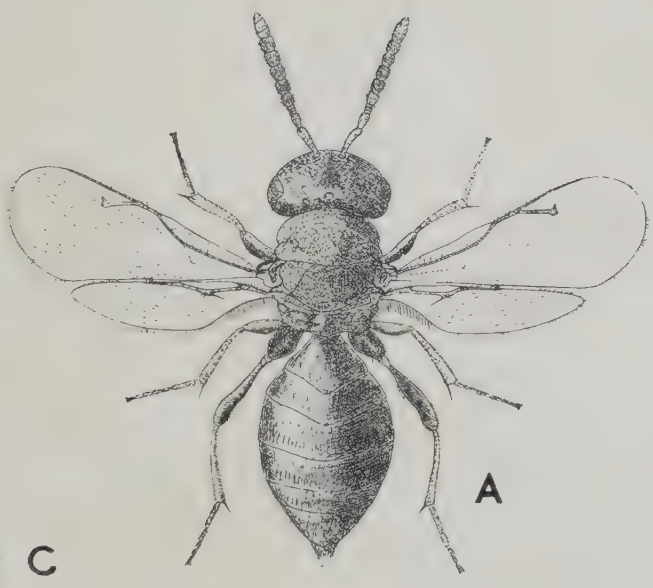
PLATE 1

Aplastomorpha vandinei

A.—Adult female.
B.—Mandible of first-stage larva.
C.—Mandible of mature larva.
D.—Mature larva.
E.—Pupa.
F.—Egg.
G.—Male adult.

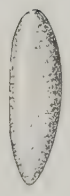


A

C

B

E



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INHERITANCE IN SWINE [1]

By EDWARD N. WENTWORTH, [2] *formerly Professor of Animal Breeding*, and JAY L. LUSH, [3] *formerly Fellow in Animal Breeding, Kansas State Agricultural College*

The genetic studies on swine described herein were begun on a rather extensive scale at the Kansas Agricultural Experiment Station in 1914, reached their maximum in the spring of 1917, at which time the senior author entered military service, and came to an end in the spring of 1918, when the junior author also assumed military duties.

With the exception of the second wild boar (Pl. 1, A), which is now the property of the Iowa State College, none of the swine used in this investigation are still living, and hence no further work can be done toward solving the unfinished problems, except by beginning once again with a new group of individuals. Since there is no immediate prospect of this being done, it is thought best to publish the results secured, together with the most probable interpretation of them, although it is frankly admitted that the data do not furnish a definite solution of the problems attacked and that in some cases they do little more than indicate the general direction for future work.

Because there have been so few carefully controlled genetic experiments on swine it is felt that there is need to make this material available for further study, even though many of the problems touched upon have not been settled. This paper, therefore, is presented as a report of progress.

PURPOSE AND PLAN OF THE EXPERIMENTS

The purpose of the experiments was to investigate the mode of inheritance of certain well-defined characters in swine, such as the shape of face, set of ears, color, mammary pattern, growth factors, and the size of litter. On all these points, except size of litter, the principal work was done on the descendent generations from crosses of a registered Berkshire boar by Tamworth and Duroc-Jersey sows. The problem of litter size was separately attacked through mating European wild boars of the Schwarzwald type to registered Tamworth and Berkshire sows. The production of a large F_2 generation and the making of suitable back crosses were planned, but only a single F_2 litter from the wild × Tamworth cross and none from the Wild × Berkshire or Berkshire × Tamworth cross had been secured when the work was discontinued. Hence the majority of the data will concern the Berkshire × Duroc-Jersey cross, from which a large F_2 generation and a considerable number of back cross individuals were obtained.

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[3] Now Animal Husbandman, Texas Agricultural Experiment Station.

The following records were kept for each pig: (1) The number of pigs in the litter in which each individual was born; (2) descriptions of color, set of ears, and shape of face, the latter character being described from three different viewpoints—length of face, dish of face, and shape of forehead; (3) a diagram of the arrangement of the mammae; and (4) growth as indicated by monthly weights.

The total number and relationship of the pigs produced in each cross were as follows:

Wild × Tamworth: This cross comprised an F_1 generation of 38 pigs produced in 5 litters out of three Tamworth sows and by two wild boars; 1 back-cross litter of 8 pigs out of another Tamworth sow and by F_1 boar; and 1 F_2 litter of 4 pigs by the F_1 boar out of a litter sister.

Wild × Berkshire: In this cross an F_1 generation of 2 litters totaling 17 pigs was secured out of 1 registered Berkshire sow by the second wild boar.

Berkshire × Tamworth: This mating produced 1 litter of 10 pigs out of a Tamworth sow by a registered Berkshire boar.

Berkshire × Duroc-Jersey: The result of this cross was an F_1 generation of 29 pigs in 3 litters out of two registered Duroc-Jersey sows by two registered Berkshire boars, an F_2 generation of 151 pigs in 17 litters out of 8 F_1 sows by an F_1 boar, 1 F_3 litter of 11, 1 litter of 5 out of an F_2 sow by the F_1 boar, and a back-cross generation of 35 pigs in 3 litters out of the Duroc-Jersey sow that was the mother of all the F_1 animals reserved for breeding, by her F_1 son.

There is no *a priori* reason to assume that similar characters in two quite distinct breeds—for example, the erect ears of the Berkshire and of the wild hog—are due to identical factor complexes. However, since it is more convenient from the standpoint of treatment, each character will be discussed in the light of the data from all available crosses.

INHERITANCE OF LITTER SIZE

Inheritance of litter size was studied in two crosses (1) wild boar by Tamworth sows and (2) Berkshire by Duroc-Jersey.

It was primarily for the study of the inheritance of litter size that the wild hog was used in this experiment, its use having been suggested in a previous study by Wentworth and Aubel (*23*).[4] This type of wild hog normally produces about 4 pigs per litter, the Tamworth 11, and the other common breeds about 8 (*23*).

Obviously this character can be fully expressed only by sexually mature females, and since so many nongenetic factors (*23*) can operate to reduce the actual number of pigs in a given litter below that which the sow is potentially capable of producing, it will not be safe to draw far-reaching conclusions from the limited data available in this experiment.

Only one F_1 sow of the wild × Tamworth cross produced any litters, and she produced but one (Pl. 1, B). The fact that her litter consisted of but four, however, agrees with the results obtained by Simpson (*11*) and is very suggestive of the dominance of the wild litter size. It may be considered a good indication that litter size in swine, like fecundity in poultry (*8*), is dependent upon certain very definite factors which can be inherited in part through the male parent, since whatever factors

[4] Reference is made by number (italic) to "Literature cited," p. 581–582.

for wild litter size this sow possessed must have been received through her sire. The average size of litters produced by the four Tamworth sows in this experiment was not up to the breed average, being only 7.67 pigs. However, four of these six litters were gilt litters, produced before their dams had reached full maturity, which may partially account for their small average size. A direct influence of the sire on the size of the litter has been demonstrated only under the condition of his previous excessive sexual use (*6*), a condition which did not apply in this case.

The data in regard to litter size in all these crosses are summarized in Table I.

TABLE I.—*Size of litters*

Description of dams.	Total number of litters.	Number of gilt litters.	Extreme size of litters.	Mean size of litters.	Standard deviation of litter size.
Pure-bred Tamworth	7	5	6 and 10	7.86	1.46± .26
F_1 wild×Tamworth	1	1	4	.....	.....
Pure-bred Berkshire	2	1	7 and 10	.....	.....
Pure-bred Duroc-Jersey	6	2	8 and 17	10.67	2.62± .51
F_1 Berkshire×Duroc-Jersey	17	8	3 and 12	8.88	2.88± .33
F_2 Berkshire×Duroc-Jersey	2	2	5 and 11	.....	.....

INHERITANCE OF SET OF EARS

Three of the breeds used in this investigation, the Berkshire, Tamworth, and wild, have erect, pointed ears of fine or moderately fine texture, and, since all of the pigs produced by intercrossing showed a very similar sort of ear, these crosses furnish no evidence as to the factors for inheritance of the ear shape. The Duroc-Jersey breed, however, is characterized by a quite different shape of ear; hence the Berkshire × Duroc-Jersey cross throws some light upon this question and will be discussed in more detail.

The Berkshire ear, in addition to being erect and pointed, is rather small and fine in proportion to the size and length of the body. It may droop somewhat with extreme age (*9*), but breaks forward at the head and not within the length of the ear.

The Duroc-Jersey ear, while not a direct opposite, offers a good contrast in several respects. It is of medium size, not so pointed, and the outer third of it breaks over sharply and droops downward. There is considerable variation in the amount of droop, but close inspection shows that these variations are rather closely correlated with variations in the general quality of the animal. Thus the large, extremely flabby ear, broken over more than breed ideals permit, is usually found on animals which show a general roughness and lack of quality. On the other hand, the extremely fine, almost erect type of ear is closely associated with a lighter limbed, neater quality type of hog. It is not known at present whether this correlation is genetic linkage or due simply to the mechanics of growth.

Both of the Duroc-Jersey sows and both of the Berkshire boars of the parental generation were typical of their breeds with respect to ear shape. The F_1 generation contained 29 pigs, and, with the exception of one boarr (Pl. 1, C, D) and one sow in the first litter, which was sired by a boar

not used subsequently, all of them, so far as could be observed, possessed perfectly erect ears, which, however, were slightly larger than on Berkshires of equal size.

Although the F_2 generation consisted of 151 individuals, only 42 had become mature enough to determine definitely the shape of ear when the work was discontinued. Of these only one (Pl. 1, F) showed a typical Duroc-Jersey ear; several showed shapes intermediate in various degrees, and the great majority showed typically erect Berkshire ears.

The Duroc-Jersey sow that was the dam of all the F_1 generation which was saved for breeding purposes was mated to an F_1 son from her first litter (Pl. 1, E). There resulted three litters totaling 35 pigs, most of which died young or were still young when the experiment was discontinued. Of the 9 which were 6 months old or more at that time, 4 had typical Duroc-Jersey ears, 1 was still undetermined, and 4 had ears completely or almost completely erect.

The following conclusions in regard to the inheritance of the set of ears are warranted by the data: The typical erect ear of the Berkshire is dominant by at least one and probably not more than three (F_2 ratio of 41 erect or intermediate to 1 of Duroc type) principal factors. There are probably a number of minor modifying factors for size and quality as well as for the amount of breaking over. Neither breed is homozygous throughout for all the factors concerned in the production of its own peculiar ear shape—that is, being pure-bred is not equivalent to being homozygous in this respect.

INHERITANCE OF SHAPE OF FACE

This character, like the shape of ear, does not complete its development until the animal is mature and is fairly constant within each of the breeds of swine.

Both the Tamworth and the wild hog are characterized by long, narrow, straight faces with almost no forehead prominence. The face of the wild hog is the more extreme in each of these respects, and the seven mature F_1 pigs all approached very closely to the face shape of their wild parent.

Among the common American breeds of hogs, the Berkshire represents the brachycephalic opposite to the wild hog in face shape, having a very short, extremely dished face and a forehead so broad and prominent as to give it a sort of pompadour appearance (Pl. 1, G). The facial angle, while varying somewhat like any other quantitative character, is fairly constant and approximates a right angle. Only two F_1 pigs from the wild × Berkshire cross matured, and both resembled the wild parent so closely that, except for color and wider foreheads, they would have been indistinguishable from the F_1's of the wild × Tamworth cross. One of them, however, also had a slightly shorter face than either his litter mate or the F_1's wild × Tamworth. Not enough data are available to indicate whether or not this was due to some variation in development.

Seven of the 10 pigs composing the F_1 generation of the Berkshire × Tamworth cross matured, and none of them could be distinguished from pure-bred Tamworths as far as face shape was concerned. This is especially surprising because Simpson (*16*), who crossed Yorkshires and Tamworths, reported that the Yorkshire face (which is quite similar in appearance in respect to dish of face to the Berkshire, though longer)

was neither dominant nor recessive, but that the faces of the resulting F_1 animals were intermediate between the two parental types. Simpson did not give the data for the F_2 generation, and none of the F_1 individuals in this experiment reproduced. Hence the number of factors responsible for the differences in face shape between these three breeds is not known. The data, however, indicate this much: Tamworth straight, long face is dominant over Berkshire short, dished face; Yorkshire dished face forms as intermediate F_1 with Tamworth long, straight face. Therefore, Yorkshire dished face and Berkshire short, dished face are apparently similar phenotypes produced by somewhat different factor complexes.

The face shape of the Duroc-Jersey is intermediate between those of the breeds just discussed, in that it is moderate in length and in dish, while the forehead and the hair which grows upon it usually slope smoothly upward and backward. In the F_1 generation of the Berkshire × Duroc-Jersey cross, the forehead in every case approaches closely that of the Berkshire type (Pl. 1, D). The face was strongly dished, but not to the degree of the Berkshire ideal, although it approached this ideal much more closely than the Duroc-Jersey type. A slight amount of variation was shown by two animals of the F_1 generation, but the dish was pronounced enough to be Berkshire in type (Pl. 1, H, I). The second of the two F_1 litters from which breeding animals were selected was much more typically Berkshire than the first. Both litters were out of the same dam, but they were sired by different boars. In every case the length of face was considerably less than the Duroc-Jersey and approached the Berkshire type. Here, too, there was some variation.

In the F_2 generation very much wider variation was observed. No practicable means of measuring any of the three elements of face shape was devised, but since they differed so distinctly, and there was so little chance of mistake, they were classified according to their resemblance to one or the other of the parental types. The face shape does not develop completely until the animal is mature, hence there are available only the data for the 42 F_2 animals which had reached maturity when the experiment was discontinued. These data are summarized in Table II.

TABLE II.—*Inheritance of facial characters in Berkshire-Duroc Jersey cross*

Facial character.	Similar to Berkshire.	Intermediate.	Similar to Duroc-Jersey.
Forehead shape	37	2	3
Dish of face	17	9	16
Length of face	21	7	14

The widest variation was in the length of face, some being longer than the typical Duroc-Jersey and some shorter than the typical Berkshire. There was some evidence of correlation between length of face and dish of face since hogs both with extremely dished and extremely long faces did not occur. On the other hand, short-faced hogs occurred both with extremely straight and with extremely dished faces. This

may be interpreted to mean that such correlation as exists is developmental or anatomical rather than genetic (linkage).

The data from this cross seems to show that the Berkshire type of face is largely but not completely dominant over the Duroc-Jersey type. Probably several factors differing in importance and in degree of dominance are responsible for the anatomical differences that determine the face shape. Certain of these factors affect certain parts of the face much more than they do other parts. If reliable means of measurement had been developed for the different aspects of face shape, it might have been possible to distinguish enough separate phenotypes to postulate a factorial analysis. In the absence of such an analysis it would be impossible even to estimate the number of factors involved, beyond calling attention to the fact that the true number can not be so large as to make analysis impossible, else the parental types would not have been recovered among such limited numbers.

RÉSUMÉ OF WORK ON SWINE COLOR (24)

In spite of the rapidity of their multiplication and the diversity of characters which they offer, very little genetic research has been conducted with swine and that which is known or seems very probable in regard to the inheritance of their colors can be stated in a very few paragraphs (1, p. 476).

The agouti color common to so many mammals is found among swine in the European wild hogs. This color, which is fundamentally a pattern of bands of different shades of sandy, red, black, or brown pigment on each individual hair is a darker, more slaty shade in the adult wild hog than is usual among other mammals. It is also characterized by being much lighter along the underline than on the sides and back. Moreover, in the wild hog the young are born with longitudinal stripes instead of being self-colored.

The European wild hog (Pl. 2A) has been crossed with the domestic breeds of hogs by Simpson in Illinois (*11, 12, 13, 14, 15, 16*) and by Herrmann and by Henseler among others in Germany as stated by Frohlich (*4, p. 219, 220–221*) but as yet there has been no very large F_2 generation produced. In the F_1 generation the striping pattern of the young and the agouti pattern of the adult have been reported dominant over the self red of Tamworths and the black with six white points of the Berkshire and incompletely recessive to two patterns—that of a white belt on a colored background and that of self white (*4, 14, 15, 18*). Simpson's data from a cross with Tamworths indicate that this wild color differs from red by a single factor.

Each individual hair of domestic swine is either white, black, or some shade of red; but within these limitations there is a wide range of colors, not only in the scrubs and grades which constitute the average market run of hogs but also between the various pure breeds. Thus there are self red, self black, and self white breeds which breed true to pattern, besides a recently established breed which is a black and white roan or "blue." Also, although there are no pure breeds of these colors, ordinary unpedigreed hogs are common which are red-and-black, red-and-white, red-black-and-white, red-and-black roan in spots, or red-and-white roan in spots. Red may be replaced by various lighter tints, ordinarily known as sandy, in any of these combinations. There appears to be no record of true albinism in the pig. If it does occur it is cer-

tainly so rare that it plays little part in the ordinary colors. Self-white breeds are fairly numerous, there being at least five known in America and one, the Edelschwein, in Germany.

The Edelschwein self white has been found dominant to the striping and agouti pattern of the wild hog (*4*), and the Yorkshire and Chester White self whites have been reported dominant in the F_1 generations over one kind of Tamworth (*12*) and Duroc-Jersey self red, and over Hampshire, Berkshire, and Poland-China (*17*) black and has produced a red-and-white roan in the F_1 of a cross with some Tamworths and Duroc-Jerseys whose self red color seems to be genetically different from the more common red color of their breeds (*19*, *20*). The black of Berkshire and Poland-China segregates in a 1 to 3 ratio in the F_2 generation, but the black pigs obtained as recessive carry more and larger white spots than the pure-bred Poland-China or Berkshire. Evidently there are some minor factors which modify the extent of this kind of black and which are inherited independently of the main factor for black or its allelomorph (*17*).

Another form of white which appears to be quite distinct, genetically, from self white and from the white spotting displayed by the Berkshire and Poland-China is the white belt, "sheet," or "list," as it is sometimes known to breeders, which is characteristic of the American Hampshire hog and, in a more extended form, of the German Hanoverian swine. This white pattern is dominant, although not always completely so, when crossed with all other colors upon which it has been tried, including even the striping and agouti pattern of the wild hog (*4*). The swine in this case were Hanoverian sows bred to a wild boar. The white belt of the Hanoverian is so extensive that usually the only parts which remain colored are the head and a part of the rump, while in the American Hampshire the white belt includes only the front legs and feet and a belt usually from 4 to 12 inches wide around the body. The two patterns are probably of the same kind genetically, except that the two breeds do not possess the same modifying factors for the width of the belt. Even in pure Hampshires, the inheritance of the belt can not be determined by less than two factors (*19*, *20*). Simpson has succeeded in producing belted red hogs by crossing the Hampshire and Tamworth and extracting reds from later generations (*12*).

A second kind of white pattern is that possessed by the Poland-China and Berkshire breeds. These are black except for white feet, a white splash in the face, and white on the end of the tail. These six white points constitute the standard color for both breeds, but quite frequently there are one or more white splashes elsewhere on the body, and occasionally an animal appears which is not pure white but a sandy color in one or more of these "white points." The complete pattern is manifestly not dependent on a few factors, since it breaks up so readily into black spotting on a white, sandy, or red ground color in crosses and is so difficult to obtain in typical form in the F_2 generation.

A possible third kind of white pattern is found in the breed known as the Spotted Poland-China. White and black, in approximately equal amounts irregularly scattered over the body, constitutes the standard color of this breed. Wright suggests (*24*) that it is doubtful whether this is really a different kind of white pattern from that of the Berkshire and Poland-China, since both of these breeds at a not very distant time were sandy white or reddish brown with large black spots. Furthermore when they are crossed their present color readily breaks up into a form

of black spotting which appears similar to that of the spotted Poland-China. Wright comes to the conclusion that the Berkshire and Poland-Chinas are genetically black-and-sandy spotted hogs in which, by selection of minor factors, sandy has been diluted to white and black has been extended to cover all the body except the six white points. Such evidence as the writers have with regard to the white points casts no light on this interpretation, but the idea seems correct for the black color.

Aside from the fact that the roans occur regularly as a part of the progeny resulting from back-crossing Yorkshire × Tamworth F_1 hogs to pure Tamworths (*12*) and in the F_1 generation of self white breeds crossed with Hampshires (*5*) nothing definite is known about the inheritance of the roaning patern. The Sapphire, the only breed making a feature of this pattern is of such recent origin that it does not yet breed completely true and hence is not suitable for analyzing this character (*7*). Several types of roans have appeared in the course of the experiments to be reported upon here but not in sufficient numbers nor with sufficient regularity to permit their analysis. As a general rule, roans appear to be either self colored or ordinarily spotted at birth, but at about weaning time hairs of another color begin to appear and soon produce the roan effect, either as a self roan or a spotted roan.

Two self black breeds are known in England but are not common in America and their color behavior in crossing is not known, although the black of the Large Black breed has been reported dominant to Tamworth red.

Hampshire black is certainly distinct, genetically, from Berkshire or Poland-China black, but whether it is equivalent to self black plus the white belt or is a third distinct kind of black is not known (*5*).

Self red breeds vary in the intensity of their pigment rather widely from a light yellow or sandy to a deep brownish red which almost approaches black. That these differences in intensity are hereditary seems probable, but no definite investigation of this point has yet been made except to find that there are two distinct kinds of red with respect to the way they react towards self white (*19*, *20*). Self red is recessive to self white, to the wild pattern, and to Hampshire black with white belt. Self red is the only type of red which is characteristic of any pure breed, but grade and scrub hogs are common which are spotted red with black or white or roan in various combinations.

This general survey of the heredity of swine color, so far as it is known, may be briefly summarized as follows:

Self white. Dominant over all other colors. Probably dependent on a single factor.

White belt. Dominant over all other colors but must depend upon more than one factor.

Immature striping and adult agouti of the wild hog. Dominant over all other colors except self white and the white belt. Dominance is not complete.

Roaning. Appears frequently among descendants of self whites by self reds, or of self whites by belted blacks.

Self black. Dominant to self red.

Black spotting on a lighter ground color. The most frequently occurring type of black in ordinary market hogs and therefore probably dependent upon a few relatively simple dominant factors.

Self Red. There are two kinds of red, genetically distinct in their behavior toward self white. Possibly the factor or factors which affect the white may be distinct from the factors for red.

COLOR DATA FROM THIS INVESTIGATION

WILD BY TAMWORTH CROSS

The color of the Tamworth is a uniform cherry red which varies somewhat in intensity within the breed but not so widely as the red of the other common red breed, the Duroc-Jersey. The color of the wild hog has already been described. All of the 38 F_1 pigs produced in this experiment were born with distinct longitudinal stripes about 1 cm. in width, composed alternately of rather light red hairs and very dark brown hairs. These stripes extended all over the backs and sides, but the bellies were a uniform light red (Pl. 1, B). Such variation as may have existed among the F_1 individuals in the regularity of striping or in the contrast between light and dark stripes was too slight to admit of description or of measurement.

All four F_2 pigs were striped in the same way except that the stripes were not quite so regularly continuous throughout the entire length of the pigs' bodies as was the case with the F_1's. These four pigs showed, however, three different belly colors, as follows: One with a uniform grayish white belly on which the hairs were apparently white, two with uniform, light reddish bellies like the F_1s and one with a similar light reddish belly on which were scattered some large black spots both in hair and skin.

The back-cross litter resulting from mating a Tamworth sow and an F_1 boar included five striped and three nonstriped pigs. Of the five striped ones, two were like the F_1's and three were faintly striped but possessed small black spots on their bodies. Of the nonstriped pigs, one was self red and the other two were red with small black spots.

Obviously this suggests single independent factors for black spotting and for the striping pattern. It also suggests that the black spotting factor may diminish the intensity of the striping pattern, because all three of the back-cross pigs which were faintly striped possessed black spots. Since none of these pigs matured, it was impossible to apply the breeding test to this latter idea, but the existence of an F_2 pig which was both intensely striped and possessed black spots makes it seem more probably that the association of faintness of striping and black spotting in the back-cross litter was due either to chance or to linkage. The factors for striping pattern and for intensity of that pattern must both come from the wild hog, while the factor for black spotting probably comes from the Tamworth, since if it comes from the wild hog the latter must also possess an inhibiting factor which prevents the spots from showing in either the F_1 or the pure wild. The occurrence of the gray-bellied pig in the F_2 generation and the absence of this type from the back-cross litter suggests that the Tamworth may possess a partially dominant factor for deep red and the F_2 pig may be explained as the segregation of the homozygous recessive to that factor. This last suggestion is supported by the fact that the wild $\times$ Berkshire F_1 pigs, to be discussed later, showed this lighter color and by the fact that the adult wild $\times$ Tamworth F_1's were a more reddish and less slaty color than the pure wild boars. This is the only actual evidence secured supporting this interpretation. No evidence was secured as to why the pure-bred Tamworth, which evidently carries a fundamental factor for black spotting, is prevented from showing that black in its own body.

WILD BY BERKSHIRE CROSS

All the 17 F_1 pigs which composed this cross were fairly uniform and striped, and, except in two particulars, closely resembled the wild × Tamworth F_1 pigs. They had much less of a reddish tinge throughout and showed a number of large black spots along the underline and on the face, ears, and legs. The lack of the reddish tinge made the dark stripes appear a blacker brown and the light stripes and underline a very light gray. The black spotting was different in appearance from that in the wild × Tamworth F_2 and back-cross pigs in two particulars; first, the spots were larger, usually being from 1 to 1¾ inches in diameter on the new-born pigs, while those on the pigs of the Tamworth cross were rarely more than ¾ inch in diameter; secondly the spots on the latter tended to be restricted to the rear half of the animal, being especially frequent on the thighs and on the underline behind the umbilicus, while the larger spots on the pigs of the Berkshire cross appeared with almost equal frequence on all parts of the underline and lower sides, although perhaps a little oftener on the lower law, the side of the face, and the lower half of the ears.

From these data it seems that the Berkshire lacks the factors determining the deeper red color which the Tamworth possesses and has either a different fundamental factor or quite different modifying factors for black spotting because this spotting shows up at once in the F_1 generation of the wild × Berkshire cross and because it is different in appearance.

BERKSHIRE BY TAMWORTH CROSS

The 10 F_1 pigs composing the only litter of this cross consisted of 8 which were red with one or more small black spots and 2 which were self-red. The red varied in intensity from a reddish gray to a dark reddish brown. Four of the 8 had many small black spots, 3 had only a few, and 1 had but a single black spot on the side of her nose. Therefore, it seems possible that there was no genetic difference between the 2 self-red pigs and the 8 with black spots in regard to the fundamental factor for black spotting, the variation being due to differences in development or differences in modifying factors for restriction of black. These pigs had, on the average, fewer and smaller spots and were of a deeper red body color than the F_1 pigs from the Berkshire by Duroc-Jersey cross to be discussed next.

BERKSHIRE BY DUROC-JERSEY CROSS

The Duroc-Jersey, as a breed, shows considerable variation in the shade of red, but the sows of the parental generation of this cross were of medium color, neither very light nor very dark. The one whose son and daughters were used to produce the F_2 generation and that was herself the dam of all the back-crossed litters was the desired cherry red. She was a good specimen of Duroc-Jersey brood sow and typical of the breed in every way except, perhaps, that she was chuffier than the breeder desires and her face was a little more dished than the average of the breed.

The 29 pigs composing the F_1 generation all possessed small black spots scattered irregularly over a yellowish red (in most cases, sandy) body color. The black spots appeared a little more numerous on the

underline and rear parts, but this was not uniformly true. The spots were all small, very few of them being over 1 square inch in extent on the new-born pig and most of them being not more than one-third as much. The red body color varied in intensity, 4 of them being described as sandy red, 2 as red with white bellies, and 1 as red with a sandy belly. Uniformly as they matured the red became lighter and, although they did not all come to the same shade of red then, it would be more accurate to describe them, when mature, as sandy hogs with numerous small black spots. Where not otherwise stated in this discussion, the color of a pig is understood to mean the color at time of birth because red and sandy were then most distinct.

Eight sows and one boar (Pl. 2, B) all of which were described as black-and-red at birth, were selected from the F_1 generation for breeding purposes and from them were produced 151 pigs (Pl. 1, J), including three which ceased development at such an early prenatal stage that their true color could not be distinguished with certainty. When classified as to the color of hair, without regard to the patterns in which these colors were arranged, the 148 F_2 pigs and the 35 back-cross pigs produced by breeding the F_1 boar back to his own dam, gave the results shown in Table III.

TABLE III.—*Color of 148 F_2 pigs of the Berkshire-Duroc-Jersey-Cross*

Color.	Number in F_2.	Number in the back-cross.
Black-and-red	47	19
Black-and-white	27	.....
Self red	14	14
Black-and-sandy	11	.....
Black-red-and-white	10	1
Black-red-and-sandy	10	.....
Sandy	7	.....
Sandy-and-white	7	.....
Black-sandy-and-white	6	.....
Red-and-sandy	3	1
Red-and-white	3	.....
White	3	.....
Total	148	35

A classification according to the presence or absence of each color separately gives the results presented in Table IV.

TABLE IV.—*Color of pigs in F_2 of the Berkshire-Duroc-Jersey-Cross, classified as to each color separately*

Color.	Number in F_2.	Ratio.	Number in the back-cross.	Ratio.
Showing black	111	3:1 (exact)	20	1:1 (approx.)
Not showing black	37		15	
Showing red	87	9:7 (approx.)	35	.....
Not showing red	61		.....	
Showing sandy	44	1:2.3	1	.....
Not showing sandy	104		34	
Showing white	56	1:1.6	1	.....
Not showing white	92		34	

The most obvious color, and the one about which there is the least chance for mistake,[5] is the black, and since it gives an exact 3 to 1 ratio in F_2 generation with such a relatively large number of individuals and such a close approximation to a 1 to 1 ratio in the back-cross, it is rather clearly evident that the presence of black color, irrespective of whatever pattern it may assume, is due in this cross to a single dominant Mendelian factor received from the Berkshire.

In a few cases it was rather difficult to distinguish accurately between red and sandy and between sandy and white, but, on the whole (Pl. 2, C), these colors segregated distinctly, and in the case of certain combinations such as black-and-red or black-and-white there was never any question. This distinctness of color was most marked when the pigs were young because some became darker as they grew older while others became lighter. There were different shades of sandy and different shades of red, but these are not considered in detail in this discussion.

The ratio of the pigs showing red to the number not showing any red is very suggestive of a 9 to 7 ratio in the F_2 generation, and the ratio in the back-cross seems to prove definitely that at least one dominant factor whose function is to produce red pigment, rather than sandy or white, is possessed by the Duroc-Jersey breed.

From the ratios of sandy to nonsandy and white to nonwhite we get definite proof only that sandy is not a heterozygous red, since the sandy pigs are so much less numerous than the reds, and that white is not due simply to an absence of factors for black and red and sandy.

The independence in inheritance of the black color and distribution of spotting with black make it possible to regard red or sandy (that is, the possession of red pigment) as the fundamental ground color of the hog. In order to get at the relation of red, sandy, and white, therefore, it may be permissible to examine them, disregarding whatever black may be shown on the bodies of the animals. If the black color is omitted from Table III, the result shown in Table V is obtained.

TABLE V.—*Distribution of red, white, and sandy colors in Berkshire-Duroc-Jersey F_2 generation*

Color.	Number in F_2.	Number in the back-cross.
Red	61	33
White	30	.....
Sandy	18	.....
Red-and-sandy	13	1
Red-and-white	13	1
Sandy-and-white	13	.....
Total	148	35

Let us first consider the relation of red and sandy. Omitting white on the theory that it is due to superimposed spotting factors, there are 87 reds (61 red, 13 red-and-white, and 13 red-and-sandy) to 31 sandy (18 sandy and 13 sandy-and-white). This misses a true 3 to 1 ratio (88½ to 29½) very slightly and suggests a single intensity factor difference

[5] Three of the pigs of the back-cross were classified as black-and-red although they possessed only one small black spot each, and in one of these cases the black was in the skin only and not in the hair growing on it. If either of these three cases has been placed in the wrong genetic classification, the correct ratio in the back-cross would still more closely approximate 1 to 1.

for red and sandy. It may be protested that the red and sandy pigs could as logically be included under sandy, but the writers have not accepted this idea because in each case where red and sandy occurred on the same hog, the sandy markings were so distributed as to indicate a failure of the intensity factor for red, just suggested, to extend to the extremities of the animal, in much the same way that the factor for restriction of black pigment in the bay horses varies centrifugally. Sandies do not appear in the back-cross to permit the testing of this hypothesis.

The data with regard to white spotting are less suggestive. Omitting the three individuals that were self white (Tables IV and V), there were 53 with white markings in the F_2 generation and 92 without. This indicates three possibilities: First, that white markings are not dominant unless dependent on the interaction of three or more factors; second, that white markings are recessive; or, third, that they are dependent on several factors, some of which are dominant and others recessive. The data are not complete enough to test any of these hypotheses. However, if the first hypothesis is correct, three or four factors are indicated. If the interaction of three factors is necessary, the expectation for 145 F_2's would be 61.2 with white markings to 83.8 without. If the interaction of four is necessary the expectation would be 45.9 with white markings to 99.1 without. The actual ratio, 53 to 92, is about half way between the two. There is no simple recessive condition that will produce this ratio, nor is there any combination of recessive conditions probable that would give the actual results. Hence the third hypothesis is most probable. In fact, three distinctly different types of white markings are readily recognizable in addition to the red-and-sandy "bicolor." The first is roaning; the second is the broken splotching of white, probably related genetically to the Berkshire "six white points," and the third is the light belly marking. The indications are that sandy may be substituted for white in many cases.

Roaning appeared in three forms. Two animals were red-and-white roan with black spots, one was sandy-and-white roan with black spots, and one was white with black-and-red roan spots. The roaning was not evident until after weaning time, and hence many of the pigs which died young also may have possessed this character. No attempt is made to analyze or explain the roaning condition.

The second form of bicolor was the irregular spotting of red or deep sandy upon a white or light sandy ground color. It always occurred in the presence of black and was readily distinguished from other colors by the fact that the red spots were always most numerous on the face, back of the head, and front part of the back. In nearly every case of this type one or both upper eyelids and eyelashes were quite red. The distinctness of this type of red spotting was not suggested until late in the investigation and consequently exact numbers are not available; but since it always occurred in the presence of black and did not appear among the back-cross individuals the suggestion is very plausible that this red spotting is genetically identical with the red or sandy splash which occasionally appears on the pure-bred Berkshire. The fact that the sandy color appears on the white splash on the face more commonly than on the feet or tail of pure-bred Berkshires supports this suggestion, and the fact that a pure-bred Berkshire practically never has all six "white points" showing sandy is another argument in favor of it. This

spotting may not be apparent in all individuals carrying it because the spots may all happen to be covered epistatically with black pigment.

The third form of bicolor appears as a red with a sandy or white belly (Pl. 2, D), or a sandy with a white belly. Black may or may not be present, but the occurrence, or at least the observance, of this character is more frequent with the nonblacks. Both the bicolor reds occurring in the back-cross and all three of the bicolor reds in the F_1 generation were of this type. The line of demarcation between the two colors may be sharp or they may blend into each other. This type of color—that is, a lighter underline than sides or back—is common to most mammals as an integral part of the agouti pattern. It is striking in the young of the wild hog, which also exhibit both (if they are genetically separate) the agouti and the striping patterns. For reasons to be discussed later it is considered probable that the three characters of lighter underline, striping pattern, and adult agouti pattern are due either to identical or to closely similar factor complexes.

A most interesting peculiarity of the F_2 Berkshire-Duroc generation was that two of its individuals, which were otherwise a light sandy with white bellies, distinctly showed longitudinal stripes of a pattern similar to that of the young of the wild hog, but not nearly so intensely colored and hence not so contrasted. They were also not so permanent and disappeared within a very few weeks after birth. In the founding of the Sapphire breed, according to McLean (7), the striping pattern appeared several times, and the stripes in some individuals persisted throughout life. Simpson also reports (13, 11) the appearance of striped individuals in crossing several breeds, of which he mentions specifically a Tamworth by Yorkshire cross and a Berkshire by Poland-China cross. This striping pattern as seen in a hog of unknown ancestry found on a farm in Wisconsin is shown in Plate 2, E. Considering everything, this phenomenon of the striping pattern in pigs is quite comparable with the appearance of barring on pigeon wings when distinct breeds are crossed and with the dorsal and shoulder stripes sometimes seen on mongrel dun-colored horses, phenomena which have been discussed since Darwin's time.

There was one litter of pigs of the F_3 generation which threw some light on this question in an unexpected manner. A white F_2 sow (which had, however, patches of black pigment in her skin although the hair growing out of these patches was quite white) when mated with an F_2 boar (Pl. 2, F) which was red with a sandy belly produced the litter of 11 pigs shown in Plate 2, G. All were some shade of sandy or red, but also all had light bellies and all were striped even more distinctly than either of the F_2 Berkshire by Duroc striped pigs. None of them showed any trace of a skin spot of black pigment. Four were classified as dark red, 5 were light red, 1 was a sandy red, and 1 was a very light sandy, almost white. The red pigment on the dark red ones was more of a brown than on any F_2 individual and their stripes were very distinct. Neither parent was observed to be striped and only the sire exhibited the lighter belly, but it is conceivable that the dam possessed the factors for both (if they are two) patterns, but did not have enough pigment in her hair for the expression of either. Although a complete factorial analysis has not yet been achieved, this much is certain; the striping pattern and the lighter underline are closely related and the factors which produce them are relatively few and simple in the mode of their expression, else such a combination would not have appeared uniformly in such a

large litter nor separately in two other litters. There is probably nothing more mysterious about this case of "reversion" than there is about any other case of the complementary action of factors. The indicated fewness of the factors affords hope for their future analysis.

The first step in investigating the nature of the genetic differences between the red, sandy, and white pigments was to discover whether the presence of the black pigment affects the other three in any way. To do this, Table III was rearranged with the black carriers separated from the nonblacks. The result, together with the numbers to be expected on the theory that the blacks and nonblacks of each combination of red, sandy, or white should give a 3 to 1 ratio, is shown in Table VI.

TABLE VI.—*The relation of black pigment to the distribution of red, sandy, and white.*

Color.	Number which also show black.	Number not showing black.	Expected blacks.	Expected nonblacks.
Red	47	14	45.75	15.25
Red-and-sandy	10	3	9.75	3.25
Red-and-white	10	3	9.75	3.25
Sandy	11	7	13.5	4.5
Sandy-and-white	6	7	9.75	3.25
White	27	3	22.5	7.5

It will be seen at a glance that the proportion of animals which had any red on them but lacked black color to those of the same color with the addition of black was almost exactly 1 to 3, or, in other words, the inheritance of the black is independent of the inheritance of the red (disregarding the pattern of the color). The numbers of the black and nonblack sandy and sandy-and-white pigs were too small for certainty, but they were far enough from the expectations to indicate a disturbing influence at work here. The number of whites was large enough and the actual ratio was aberrant enough to permit the positive assertion that the black-and-white pigs were not simply white pigs to which black had been added. There was also a difference in the appearance of the white hairs in self whites and in black-and-whites. No microscopical examinations were made, but the white of the black-and-white pigs was a more lustrous white at birth and tended much more to remain quite white all through life than that of the pigs born self white. One of the latter developed a sandy tinge at maturity, and two pigs born a very light sandy became quite white at maturity, indicating that the very dilute sandies approach very closely to the whites. The whites are certainly not albinos, for their eyes are always pigmented, and one of them had large patches of black pigment in her skin, although the hair growing out of those patches was quite white.

The second step in investigating the nature of the genetic differences between the red, sandy, and white pigments was to rearrange Table IV, classifying the bicolor reds according to the darker pigment which they showed. That the greater intensity of red represents the true pigment condition if there were no interference from bicolor factors is indicated by the fact that it was always the greater intensity of red which appeared as spots upon the lesser intensity. This classification is given in Table VII.

TABLE VII.—*The relative frequency of reds, sandies, and whites in the F_2 generation*

Color.	Blacks.	Expected blacks.	Non-blacks.	Expected nonblacks.
Red	67	62.4375	20	20.8125
Sandy	17	20.8125	14	13.875
White	27	27.75	3	2.3125

The results for the nonblacks are as good as could be desired for a 9 to 6 to 1 ratio, and at once suggest that where black is absent the color is determined by two dominant factors, each of which produce by itself a sandy color and which together produce red, while the absence of both results in white. The results for the black-carrying pigs are a moderately close approximation to a 9 to 3 to 4 ratio. They suggest that there is one factor for sandy and a separately inherited factor which intensifies sandy to red, as indicated in the discussion of Table V.

In order to reconcile the two viewpoints indicated, it is possible that the same conditions apply among blacks and nonblacks, except that there is also a dilution factor linked to black which can inhibit one of the factors causing sandy pigment, in the absence of the other, thus producing white. The factorial representation dependent on this suggestion follows:

S_1=first factor producing sandy pigment. s_1=its absence.
S_2=second factor producing sandy pigment. s_2=its absence.
D=dilution factor linked to black inhibiting S_2. d=its absence.
B=factor causing black pigment. b=its absence.

On this hypothesis the factorial representation of the zygotes of the two breeds would be as follows:

Berkshire $=\overline{BD}\ \overline{BD}\ s_1\ s_1\ s_2\ s_2$.
Duroc-Jersey $=\overline{bd}\ \overline{bd}\ S_1\ S_1\ S_2\ S_2$.
F_1 $=\overline{BD}\ \overline{bd}\ S_1\ s_1\ S_2\ s_2$.

The F_2 generation carrying black would then be:

Blacks and reds:
1 $\overline{BD}\ \overline{BD}\ S_1\ S_1\ S_2\ S_2$. 2 $\overline{BD}\ \overline{bd}\ S_1\ S_1\ S_2\ S_2$.
2 $\overline{BD}\ \overline{BD}\ S_1\ S_1\ S_2\ s_2$. 4 $\overline{BD}\ \overline{bd}\ S_1\ S_1\ S_2\ S_2$.
2 $\overline{BD}\ \overline{BD}\ S_1\ s_1\ S_2\ S_2$. 4 $\overline{BD}\ \overline{bd}\ S_1\ s_1\ S_2\ S_2$.
4 $\overline{BD}\ \overline{BD}\ S_1\ s_1\ S_2\ s_2$. 8 $\overline{BD}\ \overline{bd}\ S_1\ s_1\ S_2\ s_2$.

Blacks and sandies:
1 $\overline{BD}\ \overline{BD}\ S_1\ S_1\ s_2\ s_2$ 2 $\overline{BD}\ \overline{bd}\ S_1\ S_1\ s_2\ s_2$
2 $\overline{BD}\ \overline{BD}\ S_1\ s_1\ s_2\ s_2$ 4 $\overline{BD}\ \overline{bd}\ S_1\ s_1\ s_2\ s_2$

Blacks and whites:
1 $\overline{BD}\ \overline{BD}\ s_1\ s_1\ S_2\ S_2$ 2 $\overline{BD}\ \overline{bd}\ s_1\ s_1\ S_2\ S_2$
2 $\overline{BD}\ \overline{BD}\ s_1\ s_1\ S_2\ s_2$ 4 $\overline{BD}\ \overline{bd}\ s_1\ s_1\ S_2\ s_2$
1 $\overline{BD}\ \overline{BD}\ s_1\ s_1\ s_2\ s_2$ 2 $\overline{BD}\ \overline{bd}\ s_1\ s_1\ s_2\ s_2$

The F_2 generation lacking black would then be:

Reds:
1 $\overline{bd}\ \overline{bd}\ S_1\ S_1\ S_2\ S_2$
2 $\overline{bd}\ \overline{bd}\ S_1\ S_1\ S_2\ s_2$
2 $\overline{bd}\ \overline{bd}\ S_1\ s_1\ S_2\ S_2$
4 $\overline{bd}\ \overline{bd}\ S_1\ s_1\ S_2\ s_2$

Sandies:
1 $\overline{bd}\ \overline{bd}\ S_1\ S_1\ s_2\ s_2$
2 $\overline{bd}\ \overline{bd}\ S_1\ s_1\ s_2\ s_2$
1 $\overline{bd}\ \overline{bd}\ s_1\ s_1\ S_2\ S_2$
2 $\overline{bd}\ \overline{bd}\ s_1\ s_1\ S_2\ s_2$

Whites:
1 $\overline{bd}\ \overline{bd}\ s_1\ s_1\ s_2\ s_2$

The expectations on this basis are shown in Table VII. Aside from the superficial agreement between the expectations quoted and the actual numbers, several additional facts supporting the hypothesis may be mentioned. In both the blacks and nonblacks the reds constitute nine-sixteenths of the total number, and therefore red pigment depends upon the complementary action of two factors. From the results of the back-cross both factors must be present in the Duroc-Jersey. Also the black-and-whites constitute one-fourth of the black-carrying pigs, and therefore differ from the F_1's by one important factor. Although the numbers are small, there is one bit of additional evidence in favor of the last conclusion. A black-and-white F_2 sow mated back to her sire produced five pigs. One was all white, two were black-and-white, and two were black-and-red. One of the latter had a sandy belly. On the theory that the white of the dam differed by only one factor from the red of F_1 the expectation was that half the pigs would show white, while upon a theory that white depends upon the absence of two factors, whether black was present or not, only one-fourth of them would have shown white.

One other point in regard to color pattern remains to be discussed which is of particular interest from the standpoint of determining the color factors responsible for the large amount of black on the pure-bred Berkshire. That point is the size and extent of the black spots on the pigs showing black. In every one of the 47 black-and-red pigs the black was present in small spots and covered relatively little of the area of the pig's body (Pl. 2, H). What little unevenness of distribution of the black spots occurred in this group of animals consisted in the spots being a little more frequent along the underline and on the posterior parts of the animals. In some of the sandy-and-black animals the spots were similar to those on the black-and-reds; in others the spots were much larger and more numerous. In most of the black-red-and-whites, black-sandy-and-whites, and black-red-and-sandies the black was relatively abundant, often covering more than half of the animal. Every one of the black-and-whites (Fig. 19) possessed more black than the average of the black-and-reds, and the average amount of black on the black-and-whites exceeded that of any other group, being probably a little more than half the body surface. A large amount of variation was shown among the black-and-whites, for they varied from as little as about one-tenth black up to almost the Berkshire amount. Since no pig with absolutely perfect Berkshire markings was produced (Pl. 2, J) the evidence indicates that the Berkshire pattern is composed of the fundamental factor for black spotting, a factor for restricting or diluting the sandy pigment, and at least two or three, probably more, independent factors which operate to extend the black. The small amount of black in the F_1 generation, both of the Berkshire by Tamworth cross and of the Berkshire by Duroc-Jersey cross, and the fact that all red-and-blacks of the F_2 generation carry a similarly small amount of black are strong evidence either that there is a factor which strongly restricts black and is linked with red, or that, as Wright suggests (*24*), the competition between the two processes of pigment formation precludes the possibility of a pig possessing both an intense red and a large amount of black. The large average amount of black and the wide variation in the pattern of the black-and-white pigs indicate that the extent of the black spotting is influenced by many extension and restriction factors, even when free from the influence of the red.

CONCLUSIONS WITH RESPECT TO COLOR

The wild hog carries a single factor for the immature striping (adult agouti) pattern and a separate factor which intensifies that pattern.

There is an hypostatic factor for black spotting carried by the wild or by the Tamworth, manifested in the cross-bred specimens from either breed.

The Tamworth carries a more effective factor complex for the restriction of black than does the Duroc-Jersey. The factor for wild color and pattern is not completely dominant to the factor or factors for Tamworth red.

The Berkshire carries a single factor for black spotting and numerous independent factors for the extension of that black. Probably the Berkshire also carries dilution or restriction factors for sandy pigment and some individuals carry a factor for sandy spotting.

The Duroc-Jersey carries two factors which together produce the red color of that breed. Cumulatively one acts as an intensifier to the other. This breed lacks the factor for black spotting but possesses, probably linked to one of the factors for red, at least one factor and probably more for the restriction of black.

The relations between the various shades of red, between the various shades of sandy, and between sandy and white when black is absent are not clear.

There are three somatically distinct types of red "spotting." One is roaning, a second is an irregular spotting of red on a lighter ground color, and the third is a lighter color of belly than of sides or back. This last form is closely related to the agouti and striping patterns.

Striping in domestic swine is a rather simple case of "reversion."

It is not expected that all of these conclusions will stand in every detail the test of further research. They do, however, afford hypotheses of color inheritance in the breeds of swine investigated and offer a working basis for further research by which they may be extended and revised.[6]

DIFFERENCES IN GROWTH

The economic importance of some definite knowledge in regard to factors for rapid growth may be surmised from the extent of the practice of cross-breeding hogs in order to secure the larger size and greater vigor of the F_1 hogs for market purposes.

Only the Berkshire × Duroc-Jersey cross was extensive enough to furnish significant data upon this point. Since they were not all born at the same season nor even in the same year, it is obvious that they were not exposed to identical weather conditions nor fed rations as identical as would be desirable in a nutrition experiment. However, it was the aim always to feed the best possible ration to produce the maximum practicable gains and it is believed that the data are, on the whole, reasonably comparable.

The data for the variability of the F_1 and F_2 generations are presented separately in figure 1, where the curves show the coefficients of variability for the monthly weights for both generations from birth up to the age of 14 months.

[6] Since this was written a report (3) of the results of a cross between mule-foot and Duroc-Jersey swine has been published which agrees closely with two of the main conclusions reached in this experiment, namely, that the presence of black as contrasted to its absence is dependent upon a single factor and that the different shades of red are due to the interaction of a few independent factors which do not affect black. The black of the mule-foot swine behaved like that of the American Hampshire as far as it has yet been analyzed, but whether the two are genetically identical is still undetermined.

Table VIII presents the data from which these curves were constructed. The decrease in the number of individuals indicated in the earlier months was due both to deaths and to the fact that many of the animals were still young when the experiment was discontinued. After the eighth month the decrease was due mostly to sales. This, by culling out the larger individuals, may have been responsible for a certain amount of decrease in variability of the F_2 generation but was without effect upon the curve of the F_1 generation for there were no sales from it and the deaths seemed to be normally distributed among the large and small animals.

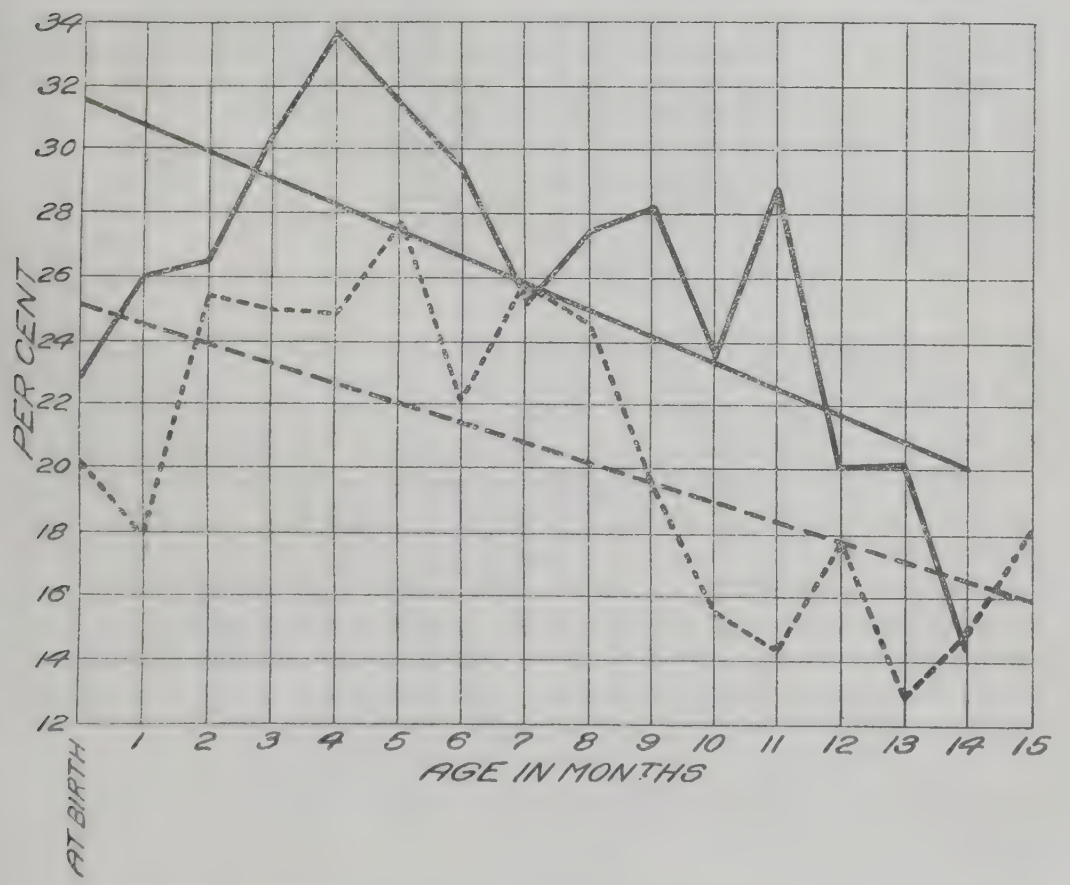

FIG. 1.—Coefficients of variability for the monthly weights of the F_1 and F_2 generations of the Berkshire × Duroc-Jersey cross. The solid line represents curve and fitted straight line for the F_2 generation, the dotted line the curve for the F_1 generation, and the broken line the fitted straight line for the F_1 generation

TABLE VIII.—*Variability of the F_1 and F_2 generations.*

Age.	F_1				F_2			
	Number of individuals.	Mean weight.	Standard deviation.	Coefficient of variability.	Number of individuals.	Mean weight.	Standard deviation.	Coefficient of variability.
		Pounds.				*Pounds.*		
Birth	13	2.46	0.498	20.3	136	2.4	0.547	22.77
One month	20	13.2	2.36	17.88	102	10.07	2.62	26.02
Two months	20	25.65	6.55	25.54	83	18.89	5.0	26.47
Three months	19	35.05	8.79	25.08	56	32.88	10.04	30.54
Four months	18	47.72	11.89	24.92	55	46.29	15.52	33.53
Five months	17	61.94	17.14	27.67	51	64.71	20.31	31.39

TABLE VIII.—*Variability of the F_1 and F_2 generations*—Continued.

Age.	F_1				F_2			
	Number of individuals.	Mean weight.	Standard deviation.	Coefficient of variability.	Number of individuals.	Mean weight.	Standard deviation.	Coefficient of variability.
		Pounds.				*Pounds.*		
Six months	15	86.0	19.01	22.1	48	87.54	25.76	29.43
Seven months	15	115.6	30.04	25.99	43	113.0	28.58	25.29
Eight months	13	135.61	33.18	24.47	43	130.46	36.0	27.59
Nine months	13	164.31	32.32	19.67	32	137.19	38.71	28.22
Ten months	13	189.23	29.19	15.43	33	159.27	37.71	23.68
Eleven months	12	197.25	28.38	14.39	19	166.79	48.12	28.85
Twelve months	12	218.92	38.95	17.79	11	181.55	36.5	20.1
Thirteen months	11	225.36	29.05	12.89	9	202.33	40.83	20.1
Fourteen months	10	229.5	34.1	14.86	7	223.57	32.25	14.43
Fifteen months	10	226.7	41.1	18.13	2	195	.....	.....
Sixteen months	10	242.8	41.1	16.92	.....	.....	.....	.....

The outstanding fact of genetic importance is that the variability of the F_2 generation is distinctly greater than that of F_1. That, to be sure, is what was to be expected from the genetic standpoint, and it is in agreement with the experience of practical breeders. It is definite proof of two facts: First, some of the factors which have the power to stimulate growth are not identical in the Duroc-Jersey and the Berkshire breeds; second, there is some degree of homozygosis for these growth-stimulating factors within the limits of each breed. To what extent being pure-bred from the standpoint of the breed registry society indicates homozygosis for these growth factors; whether the number of factors involved can be determined; whether the differences of growth indicate differences in the identity of the factors or merely differences in the combinations; and whether these factors are so linked in groups as to make recombination of the desirable ones impossible, or at least impracticable—these are all interesting questions which these data raise but are too meager to answer.

Other interesting questions which are not, however, primarily genetic, relate to the shape of the curve. The maximum variability seems to be reached at or shortly following weaning time and coincides with the period of the lowest mortality. Does this mean that the best time to select breeding stock for size and early maturity is when they are from 3 to 6 months old? Of course it was impracticable to keep the hogs, which were not desired for breeding purposes, until they were 2 or 3 years old, and therefore there is still left open the question as to whether the hogs would not have finally become more uniform in size; that is, as to whether the differences were in potential size or in earliness of maturity.

Plate 2, K, is a photograph of one of the F_2 litters most extreme in its range of variation in growthiness. These four individuals had received identical treatment, and none of them had ever been recorded as sick, yet when this photograph was taken, when they were being marketed at the age of 11 months, the weights were, from left to right, 221, 293, 90, and 170 pounds. Possibly more animals are born runts than is commonly believed.

MAMMARY PATTERN

This subject was treated by the senior author in a detailed study (21) based upon many more data than are available in this experiment. Therefore, the present data will merely be briefly summarized in so far as they bear upon the following points, which are discussed in detail in the original study.

SEAT OF THE GREATEST VARIATION

The frequency of the occurrence of the nipples and the variations in their positions are presented in Table IX. The method of counting the position of the mammae is the same as that used in the original paper.

TABLE IX.—*Number and position of nipples of all pigs*

	Number and frequency of pair—						
	1	1½	2	2½	3	3½	4
Number of nipples....	536	3	466	39	495	6	498
Frequency mammae..	98. 52	. 56	86. 3	7. 22	90. 92	1. 11	92. 22
Frequency variations.	1. 48	.....	13. 7	.....	9. 07	.....	7. 78

	Number and frequency of pair—						
	4½	5	5½	6	6½	7	7½
Number of nipples....	16	423	46	268	11	124	0
Frequency mammae..	3. 29	86. 32	9. 39	81. 21	8. 33	93. 93	.....
Frequency variations.	.....	13. 68	.....	18. 79	.....	6. 06	.....

	Number and frequency of pair—		
	8	8½	Inguinal.
Number of nipples.....	3	1	536
Frequency mammae.....	75	25	98. 53
Frequency variations.....	25	.....	1. 47

The second wild boar himself possessed but four pairs of mammae and the number of mammae in his offspring was so uniformly less than in animals of the domesticated breeds that the wild boars and all their descendants, which are included in Table IX, are grouped separately in Table X.

TABLE X.—*Number and position of nipples of wild boars and descendants*

	Number and frequency of pair—						
	1	1½	2	2½	3	3½	4
Number of nipples....	111	.....	99	6	104	1	96
Frequency mammae..	99. 1	.....	88. 39	5. 36	92. 86	. 9	88. 89
Frequency variations.	. 9	.....	11. 61	.....	7. 14	.....	11. 11

TABLE X.—*Number and position of nipples of wild boars and descendants*—Continued.

	Number and frequency of pair—				
	4½	5	5½	6	Inguinal.
Number of nipples	7	58	2	7	111
Frequency mammae	6. 48	85. 29	2. 94	87. 5	99. 1
Frequency variations		14. 71		12. 5	. 9

Evidently the smaller number of mammae possessed by the wild race is inherited to some extent, but whether as an independent character or because correlated with other body structures is not disclosed by these data.

Using the data in Table VIII and arranging the pairs in the order of frequency of variation, the following sequence results: Sixth, second, fifth, third, fourth, seventh, and inguinal. (The eighth was discarded because of small numbers.) This sequence duplicates exactly that found in the original study, except that there was no seventh pair in those animals.

The rank of the point of most frequent appearance of the triangle pattern is as follows:

	Percentage of total.	Percentage of pigs of the wild cross.
Between fifth and sixth	9. 39	2. 94
Between sixth and seventh	8. 33	
Between second and third	7. 22	5. 36
Between fourth and fifth	3. 29	6. 48
Between third and fourth	1. 11	. 9
Between first and second	. 56	

This agrees almost exactly with the previous results. (The triangle at 8½ is not included because of the small numbers.)

The rank of the point of most frequent suppression of a nipple was as follows:

	Percentage of total.	Percentage of pigs of the wild cross.
Second pair	5. 33	6. 25
Fourth pair	3. 89	3. 70
Sixth pair	1. 82	
Fifth pair	1. 02	1. 47
First pair	. 74	. 89
Third pair	. 55	. 89
Inguinal pair	. 36	. 89

While this does not agree exactly with the previous results, still no pair is further than two places from its original rank, and, in general, the order is similar.

RELATION BETWEEN NUMBER OF MAMMAE AND ASYMMETRICAL VARIATIONS

To determine the relation between the number of mammae and the frequency of the suppressed nipple or the triangle variations, the animals were grouped as shown in Table XI. Twelve animals possessed both variations and are counted twice.

TABLE XI.—*Relation between number of mammae and frequency of suppressed nipple*

	Pair No. 4.	Pair No. 5.	Pair No. 6.	Pair No. 7.	Pair No. 8.	Pair No. 9.
Number of animals of each class with all even pairs of mammae	2	26	42	51	5	0
Per cent	100	96. 3	51. 85	36. 43	11. 36	.....
Number of animals of each class with triangle	.....	.....	21	68	20	2
Per cent	.....	.....	25. 93	48. 59	47. 5	67
Number of animals of each class with suppressed mammae	.....	1	18	21	19	1
Per cent	.....	3. 7	22. 22	15. 0	45. 45	33

The increased percentage of animals with two types of variations among the animals with a larger number of mammae speaks for itself.

INHERITANCE OF THE TWO FORMS OF VARIATION

Nothing could be learned in regard to this point from these data because of the smallness of numbers and because the only boars with any considerable number of offspring—the second wild boar and the F_1 boar of the Berkshire × Duroc Jersey cross—lacked both variations. The only female having a very large number of offspring—the F_1 Duroc-Jersey sow—possessed the suppressed mammae herself, but produced some offspring having an even pattern, others having a triangle, others with a suppressed mammae, and others with both variations when mated to her son with an even pattern, and still others with a triangle when mated to a Berkshire boar whose pattern is not recorded.

RUDIMENTARY MAMMAE TO THE REAR OF THE INGUINAL PAIR

The possession of this pair of mammae which is low upon the scrotum of the male or upon the inner part of the rear thighs of the female, has been described (*21*) as a sex-limited character due to a factor which is dominant in males but recessive in females, and as a sex-linked character (*22*) for which the male is the simple × sex. However, it appears that the latter interpretation is faulty in view of certain data presented in the original article (*22*) in which this interpretation was advanced. These data are those which show that there resulted from the mating of boars with rudimentaries to sows with rudimentaries, offspring in the ratio of two boars possessing rudimentaries to one female possessin

them to one female lacking them. The occurrence of the female lacking rudimentaries appears inexplicable upon the sex-linked basis, and to that extent the original sex-limited interpretation appears more nearly correct. This is especially true because the only published evidence against it has been the purely negative evidence of failure to find a boar homozygous for the factor for rudimentary mammae. The sex-linked interpretation is therefore abandoned.

However, the data disclosed in the present experiments do not completely support the sex-limited interpretation, although clearly indicating that the character is in some way involved with sex. The authors are somewhat at a loss to know how to regard these data. The aberrant individuals indicated in Table XII were recorded by three separate persons, none of whom was the senior author, who had had the greatest experience in recognition of the character. The fact of his absence in the military service made it impossible for him to check the records, hence they are presented for what they are worth in the belief that their publication is less open to criticism than their suppression. With the exception of one female recorded as possessing rudimentaries which is discussed later, it will be noted that the aberrant animals possibly result from errors of omission rather than of commission, hence it is probable they represent a lack of uniformity in observation among the different people connected with recording the data of the study. The data as recorded from the different types of matings are given in Table XII.

TABLE XII.—*Distribution of rudimentaries in the offspring*

Character of parents.	Number of matings included.	Number of litters included.	Males.		Females.	
			With.	Without.	With.	Without.
Both with rudimentaries	9	16	56	21	33	37
Both without rudimentaries	4	5	.....	12	.....	22
Male with, female without	1	4	8	11	1	10
Male without, female with	1	2	1	4	1 (?)	11

The numbers in bold-faced type are those which were unexpected according to the original sex-limited interpretation of the inheritance of this character. The female with rudimentaries produced in the fourth type of mating is quite certainly wrongly classified, for the record shows her to have only one rudimentary and a mammary pattern so irregular that it is probable the one described as a rudimentary is really the last mamma of the abdominal series.

Omitting the questionable female from consideration, the following discussion is offered of the results in Table XII. Except for one litter in the first type of mating, all litters in the first and third types were sired by the same male, the F_1 Berkshire-Duroc-Jersey boar, and the dam of the one mating of the third type and the dams of seven of the nine matings of the first type were all full sisters to each other and to the boar. In spite of this close relationship, these matings when considered individually do not show a normal range of distribution in the ratios of males with rudimentaries to males without. Instead, four of the nine matings in the first type show approximate equality of males

with and males without rudimentaries. The other five matings show altogether only 2 males without the rudimentaries and 32 with the character. The data do not furnish critical evidence as to whether there are different genotypes among the females or whether there is merely a quite abnormal distribution. The numbers, of course, are not large, but they approximate so closely a simple 3 to 1 ratio for the males and a simple 1 to 1 ratio for the females from the first type of mating that they suggest strongly that the true explanation must be simple.

All the animals of the first and third types were entirely Berkshire and Duroc-Jersey in their ancestry. Three matings of the second type were entirely Tamworth and wild in their ancestry, and the fourth mating of this type was between a pure-bred Berkshire boar and a pure-bred Tamworth sow. The only mating of the fourth type was between a pure-bred Berkshire sow and the wild boar. Hence it appears at least possible that there may exist differences between the breeds in respect to the factor complex which cause this pair of mammae to be present. Thus, the matings of the second and fourth types, which produce the small percentages of progeny with rudimentary mammae (or none at all) include all the matings in which either Tamworth or wild blood is involved. Likewise all the matings in the first and third types involve only Duroc-Jersey and Berkshire blood, most of them being matings between F^1 individuals. It is worthy of note that the original theory of sex-limited inheritance of this character was based almost entirely upon data obtained from high-grade or pure-bred Duroc-Jersey animals (*21*).

It is not possible from these data to show clearly which of the expressions of the character is the dominant one. From the evidence of the matings of the first and second types it would seem that the possession of the rudimentaries was the dominant form of the character, since parents lacking them produce only offspring like themselves while parents possessing them produce both kinds of offspring. However, such an hypothesis entirely fails to explain the great preponderance of progeny without the character in the matings of the third and fourth types.

Since the numbers of animals in this study are so much smaller than in those previously reported by the senior author, and since there was so much confusion incidental to the entering into the military service of three of the four men connected with the records on this work, it is believed best not to consider the evidence critical until further data are secured which either support or discount the present records.

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PLATE 1

A.—The second wild boar used in these experiments. (Courtesy of the Iowa Agricultural Experiment Station.)

B.—Two pure-bred Tamworth sows and a litter out of one of them by a wild boar. The pigs are about 6 weeks old.

C.—The F_1 boar whose ears were not erect. Not used in breeding. Berkshire × Duroc-Jersey cross.

D.—A Berkshire × Duroc-Jersey F_1 barrow showing the almost complete dominance of the Berkshire shape of ear and face.

E.—The Duroc-Jersey sow that was the dam of all the F_1 breeding animals in the Berkshire × Duroc-Jersey cross.

F.—Two F_2 individuals showing the segregation of ear shapes. Berkshire × Duroc-Jersey cross.

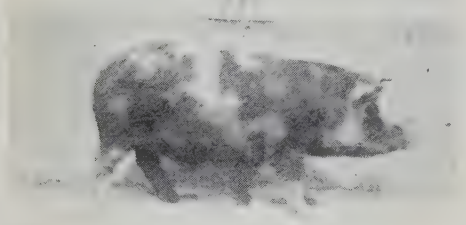
G.—The "pompadour" Berkshire type of forehead as seen on an F_2 individual of the Berkshire × Duroc-Jersey cross.

H.—An F_1 barrow with exceptionally straight face. Berkshire × Duroc-Jersey cross.

I.—A particularly long-faced individual. F_1 generation of Berkshire × Duroc-Jersey cross.

J.—Two F_2 litters of the Berkshire × Duroc-Jersey cross, showing the diversity of colors produced.

A



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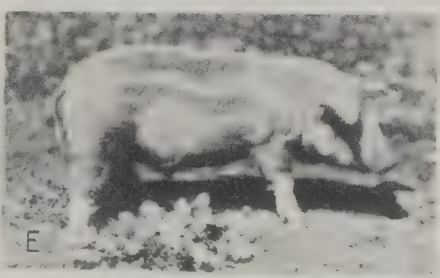
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PLATE 2

A.—The F_1 wild × Tamworth boar and the second wild boar.

B.—The F_1 boar, sire of all the F_2 pigs of the Berkshire × Duroc-Jersey cross.

C.—A self-red F_2 barrow. Berkshire × Duroc-Jersey cross.

D.—An F_2 barrow, red with a sandy belly when born, white when mature. Berkshire × Duroc-Jersey cross.

E.—A common type of "reversion." A striped pig of ordinary mixed domestic ancestry. (Courtesy of the Department of Genetics, University of Wisconsin).

F.—An F_2 boar, red with a sandy belly. Berkshire × Duroc-Jersey cross.

G.—An F_3 litter. Eight pigs of the 11 which showed stripes and light bellies. Berkshire × Duroc-Jersey cross.

H.—A red-and-black F_2 pig showing the average amount of black. Berkshire × Duroc-Jersey cross.

I.—A black-and-white F_2 pig showing the average amount of black. Berkshire × Duroc-Jersey cross.

J.—The black-and-white F_2 pig which most nearly approached the Berkshire standard as to the amount of black. Berkshire × Duroc-Jersey cross.

K.—Four litter mates from the F_2 generation. Note the variation in size. Berkhire × Duroc-Jersey cross.

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EARLY VIGOR OF MAIZE PLANTS AND YIELD OF GRAIN AS INFLUENCED BY THE CORN ROOT, STALK, AND EAR ROT DISEASES[1]

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PURPOSE OF THE PAPER

The purpose of this paper is (1) to emphasize the important relation existing between early vigor and yield of dent (*Zea mays indentata*), (2) to call attention to the reduction in early vigor of corn plants grown from seed infected with one or more of the corn root and stalk rot pathogenes or seed susceptible to the root and stalk rots, and (3) to present data bearing directly on the correlation between this reduced early vigor and reduced yield, with special reference to reduced yield of marketable corn.

A study of any field of corn, especially during the first few weeks after the plants have appeared above the ground, reveals striking differences in plant vigor. These differences in early vigor may be caused by many factors and may express themselves in both the size and appearance of the plants.

REVIEW OF LITERATURE

Darwin (7)[3] found that maize plants grown from self-fertilized seed were slower growing and less vigorous than plants grown from cross-fertilized seed. Beal, of the Michigan Agricultural Experiment Station (*28*), in 1877-1882 reported an increase in both vigor and yield in first-generation hybrids when certain unrelated varieties were crossed. He urged the commercial utilization of this increased vigor as a method of increasing yields. The results of the early experiments of Beal were confirmed by other workers. Collins (*5*, *6*) found that many crosses between diverse types of maize resulted in greatly increased vigor, a wider range of adaptability, and increased productivity.

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[3] Reference is made by number (*italic*) to "Literature cited," p. 628–629.

Studies by East (*9*, *10*), Shull (*36*), East and Hayes (*11*), Jones (*24*), and East and Jones (*12*), with inbred strains or biotypes, showed that one of the effects of inbreeding in maize was a reduction of vigor and yield. Crossing unrelated inbred strains greatly increased vigor and yield. They (*11*) state that "inbreeding always reduced the yield of seed and the height and delayed the time of flowering." These workers pointed out that reduction in vigor due to inbreeding may or may not be accompanied by pathologic symptoms (*11*). An illuminating discussion on inbreeding and hybrid vigor, or heterosis, together with an extensive bibliography, is given by East and Jones (*12*).

In his investigations of the chlorophyll inheritance in maize, Lindstrom (*27*) observed "a general correlation between chlorophyll factors and plant growth."

Ewing (*13*) obtained the following correlations with Funk Yellow Dent:

Weight of grain and diameter of stalk	+0.393	±0.020.
Weight of grain and breadth of leaf	+ .314	± .021.
Weight of grain and height of seedlings	+ .219	± .037.
Weight of grain and height of mature plants	+ .203	± .025.

He expressed the belief that very little use could be made of correlation in practical corn breeding. He stated that there were rare cases in which the coupling of unit characters may assist the breeder in making selections at an early period of growth, but that the correlation in the fluctuating variability of two different characters is not likely to prove of much assistance.

Pearl and Surface (*30*) made a study of growth and variability in sweet corn in the summer of 1908. Growth measurements of a large number of plants were made at intervals of three and one-half days, beginning on June 11 and continuing until growth was completed. They found that the relative variability of height decreased as the season advanced and was on the average about 10 per cent lower in the matured plants than at the beginning of the season. During the tasseling period a marked increase in variability was noted, followed by a gradual decline. Their experiments demonstrated that plants relatively vigorous or weak at the beginning of the season tend to retain this comparative vigor. They regarded the differences in manner of growth as due to independent Mendelian factors distributed at random in the population of open-fertilized plants.

Reed (*31*) studied the growth of a number of sunflower plants (*Helianthus annuus* L.). Measurements of the plants were made in centimeters at 7-day intervals during the greatest period of growth. The plants were grouped in four equal numerical divisions called quartiles, on the basis of size. The season's study of the plants in these quartiles showed a well marked tendency to remain in a given quartile during the entire period of growth. Plants small at maturity generally had been small from the beginning and those large at maturity had a well-marked superiority from the start. Reed (*31*) found, as did Surface and Pearl (*30*), that height was determined by independent height factors distributed at random through the segregating population which he studied.

Hughes (*22*) made extensive measurements of plants during two years, involving 828 ear rows. He found that plants highest at the first measurement made the most rapid average height increment per plant and produced the greatest yield. Plants from horny kernels made a more rapid height increment than plants from starchy kernels and also produced

the greater yields. Kyle (*26*) found that corn plants making a vigorous early growth gave greater yields than those that were weak in their early stages of development. Grantham (*14*) studied the development and maturity of 100 hills of corn where a weak plant was growing with a vigorous one. From 50 of the hills he removed the weak plant and from 50 others he removed the vigorous plant. Measurements taken at different dates showed that the vigorous plants made the most rapid early growth and the greatest yield of shelled corn per plant. The vigorous plants tasseled on the average about nine days earlier than the weak plants.

From time to time various investigators have attributed the cause of slow and irregular growth in maize to the influence of pathogenic organisms. Burrill (*3*) in discussing a bacterial disease of corn said:

> The first indication of the disease in a field of corn, as noticed in ordinary observation, is the dwarfed condition of the young plants.

Duncanson (*8*) noted that diseased plants were dwarfed, slender, and generally unhealthy. Stewart (*37*), working with sweet corn (*Zea mays saccharata*), noted that—

> usually, the small plants are the first to succumb to the disease, which fact suggests that the disease may be the cause of their slow growth. This suspicion was confirmed by microscopic examination. Plants green and apparently healthy except for their small size, were found to contain a considerable quantity of the bacterium in their vessels, while in the larger, more vigorous plants, the bacterium could not be found. However, in wet weather the bacterium may sometimes be found in quite vigorous plants.

Selby (*34*) noted conditions similiar to those described by Burrill. Later he (*35*) stated that the root rot of corn caused a dwarfing of the plants and a failure of the stalk to produce an ear. Hoffer and Holbert (*15*) pointed out that the use of diseased seed corn was one of the causes of high percentages of blighted and stunted plants and subsequent unsatisfactory yields. Rosen (*32*), in describing a bacterial disease of corn, noted that the diseased plants could be recognized easily by the short, stunted nature of the stalks. Holbert, Dickson, and Biggar (*16*) conducted experiments in which half of the hills were inoculated at planting time with a pure culture of *Gibberella saubinetii* (Mont.) Sacc. They summarized their results as follows:

> In the inoculated hills germination was lowered, early growth retarded, relative vigor during the season reduced, and grain production lessened.

The pathogene with which they were inoculated was readily recovered from the diseased plants during the seedling stage. They stated further that—

> Plants, strong or weak in the early stages of growth, had a tendency to retain this relative vigor throughout the season. Average grain production was directly correlated with rate of early growth and early vigor.

Trost and Hoffer (*38*) found that starchy ears of corn of certain dent varieties produced larger numbers of weak-growing and root rot susceptible plants in the field and yielded less per acre than the plants from ears of more horny composition, irrespective of whether or not the starchy kernels were infected with *Fusarium* spp. before planting.

Baker (*2*), Butler (*4*), Palm (*29*), Rutgers (*33*), and others working in the Orient have found that the downy mildews (*Sclerospora* spp.) in attacking young maize plants, cause a serious stunting in their early growth. Plants so affected seldom produce grain. A complete description of this disease is given by Weston (*39*).

EXPERIMENTAL METHODS

The investigations here reported include data on approximately 153,000 corn plants grown (1) in experimental plots in the vicinity of Bloomington, Ill., in the four years 1918 to 1921, on uniform, well-drained, fertile soil, classified by the Illinois Soil Survey (*18*) as brown silt loam; (2) on the Agronomy South Farm of the Illinois Agricultural Experiment Station, University of Illinois, Urbana, a brown silt loam (*19*); and (3) on an experimental plot near Yates City, Ill., also a brown silt loam (*20*). The seed, representing varieties differing in length of growing season, was all selected before a killing frost, carefully dried, and properly stored.

The term "relatively disease-free," wherever used in the tables, refers to seed showing no symptoms of infections by any of the root and stalk rot pathogenes, including species of Gibberella, Fusarium, and Diplodia. This information was obtained by testing representative samples on the limestone-sawdust table germinator described by Holbert and Hoffer (*17*). The term "diseased" refers to seed showing such infections when tested by this same method. The testing of every kernel of a great number of seed ears has given abundant evidence that usually not all the kernels on ears classed as "relatively disease-free" are actually free from infections. Nor is every kernel on ears classed as diseased actually infected; in fact, the percentage of infected kernels in any of the diseased seed lots used usually was less than 50. The term "nearly disease-free" refers to seed lots as nearly free from infection as it was possible to have them.

The plots were hand planted in hills 42 inches apart each way at the rate of three kernels per hill. Great care was exercised throughout the season to avoid mechanical injury of the plants during cultivation and to guard against ravages of insect pests and rodents, but no attempt was made either to thin to a uniform stand or to correct for differences in stand. When there was any appreciable damage due to these causes the experiments were discarded entirely. Both acre yields and individual plant yields were recorded in terms of the weight of dry shelled corn. Acre yields have been reduced to a uniform moisture basis.

Measurements and other readings taken in connection with these investigations were not made on plants selected at random; every individual plant in each of the various experiments herein reported was measured and the measurements were included in the data. Plant height was measured to the tip of the tallest leaf. Stalk circumference was measured at the middle of the first internode above the ground surface.

Each plant was studied in the early growth stages from the standpoint of vigor and classified as either vigorous, semivigorous, or weak. This classification was based on the general appearance of the plant, including size, color of foliage, apparent freedom from disease symptoms, and other characters usually associated with vigor. With few exceptions these records were made about 30 days after planting.

For three years corn inoculation studies have been conducted in cooperation with Dr. James G. Dickson, of the Office of Cereal Investigations, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station, to investigate further the relation of *Gibberella saubinetii*, the common wheat-scab organism (*23*), to the root and stalk rots of corn. It has been found that this organism, under conditions to be discussed in other papers, may be very active in reducing early vigor of corn plants. A limited number of these data are included in this paper to show

that reduced early vigor, as a result of artificial inoculation with one of the root and stalk rot pathogenes, also is directly correlated with reduced yield. The term "inoculated," as used in the tables, refers to corn grown from seed inoculated at planting time with *Gibberella saubinetii*, the inocula being prepared by Dickson. The term "uninoculated" refers to corn in contiguous plots of equal size grown from similar seed not inoculated.

The yields from experiments conducted in 1920 and 1921 were separated into "marketable" and "unmarketable" grades. The latter grade consisted of nubbins, rotted or partly rotted ears, and light chaffy ears. Ears that were less than half the length of an average large-sized ear of the variety were classed as nubbins. Larger ears also that were poorly filled were classed as nubbins. The term "rotted ears" included any that showed an appreciable extent of rot due to Diplodia, Fusarium, or other causes. The chaffy ears were those with the grains imperfectly matured so that the kernels were thin and loose on the cob. Chaffy ears that also showed signs of rot were classed as rotted. (See Pl. 2, 3, and 4.)

The term "infested" soil, as used in this paper, refers to soil known to be infested with one or more of the root and stalk rot pathogenes. In every case where this term is used the soil had been cropped to corn or wheat for the previous three years. The wheat had been heavily infected with scab (*Gibberella saubinetii*), and the corn crops were known to be badly diseased.

The term "clean" soil, as used in this paper, refers to soil reasonably free from any of the root and stalk rot pathogenes. This soil had not been cropped to corn or wheat for at least 10 years.

EXPERIMENTAL DATA

The experimental data cover numerous and extensive studies on the relation of the vigor of corn plants in the early stages of their growth to their subsequent yield, under different conditions. The variables include differences in infection of the seed by rot-producing pathogenes, differences in physical character of the kernels, and differences in the soil, in character, treatment, and quantity of infestation.

COMPARISON OF EARLY HEIGHT AND YIELD OF TRANSPLANTED SEEDLINGS

During the summer of 1918 a number of corn seedlings were transplanted to the field and studied in order to compare the performance of apparently disease-free and diseased seedlings. The seedlings were grown on the limestone-sawdust table germinator until the plumules were approximately 3 inches long. At this time those selected for further study were transferred to small paper boxes containing moist soil free from any of the corn rot pathogenes. One day later the boxes and seedlings were transferred to the field, the healthy and diseased seedlings being placed in alternate hills 42 inches apart. Both types of seedlings used in these studies had the same height and relative vigor when taken from the germinator. These seedlings were so selected that they represented adjacent kernels on artificially cross-pollinated ears from apparently healthy plants. The field in which these studies were carried on had not grown a crop of corn for at least 10 years.

The healthy seedlings suffered no ill effect from transplanting and made steady growth in height from the time they were transplanted.

On the other hand, all of those in the diseased group remained stationary in height for a period of 10 to 15 days. At the end of that time some of the diseased plants began growing and continued to progress at about

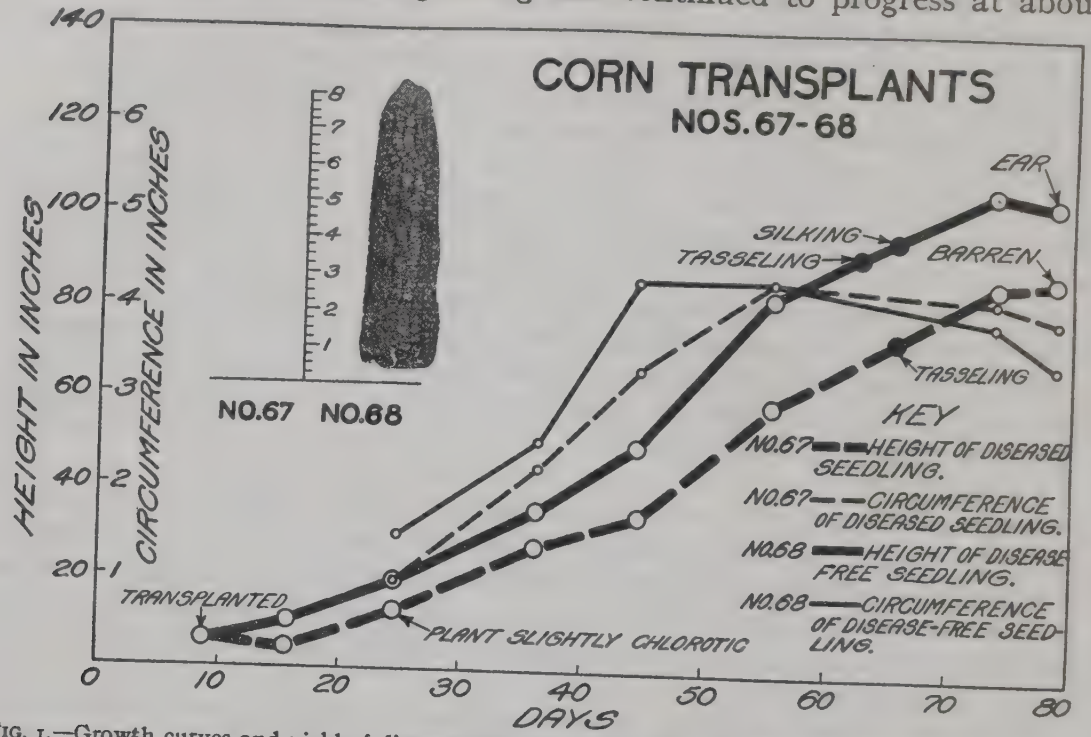

FIG. 1.—Growth curves and yield of diseased (No. 67) and healthy (No. 68) corn transplants. During the first week there was an actual decrease in height of the diseased transplant on account of the blighting of the leaves. During the following three weeks the diseased plant recovered somewhat from the severe attack of the root and stalk rot pathogenes and grew at about the same rate as the healthy plant, but after that, it failed to make as rapid progress. The diseased transplant required about 10 days longer to attain its maximum circumference. It failed to produce any grain, whereas the healthy transplant produced the good ear shown.

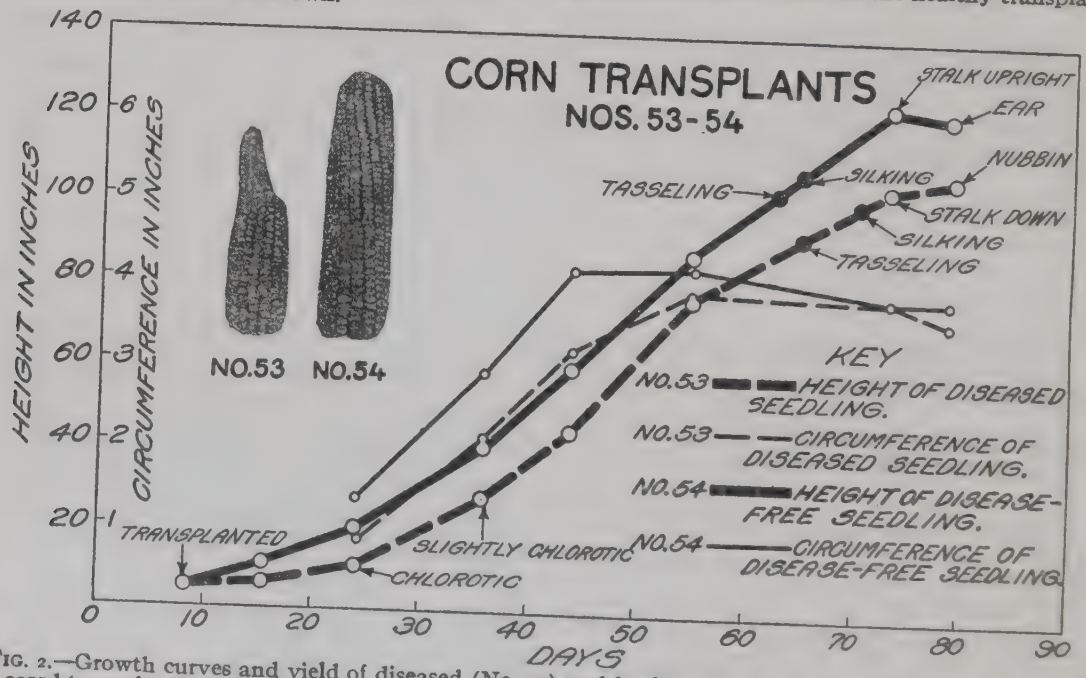

FIG. 2.—Growth curves and yield of diseased (No. 53) and healthy (No. 54) corn transplants. The diseased transplant failed to make any progress in height during the first week or 10 days, after which it grew at a rate parallel with that of the healthy transplant. The diseased transplant was considerably later in attaining its maximum stalk circumference, in tasseling, and in silking and produced a considerably smaller ear.

the same rate as the healthy plants with which they were compared (fig. 1 and 2). Some of the diseased plants finally attained a height equal to that of the healthy plants (fig. 3) but they were later in tasseling

and silking and did not yield nearly so much grain as the healthy plants. Others, however, grew very slowly and finally succumbed to the attacks of the parasitic organisms with which they were infected.

A summary of these data is given in Table I. It will be observed that the diseased transplants never recovered sufficiently from the stunting in growth during the first 15 days to produce a normal yield of grain. In some cases the diseased plants bore a cob of normal length but on it there were only a few kernels.

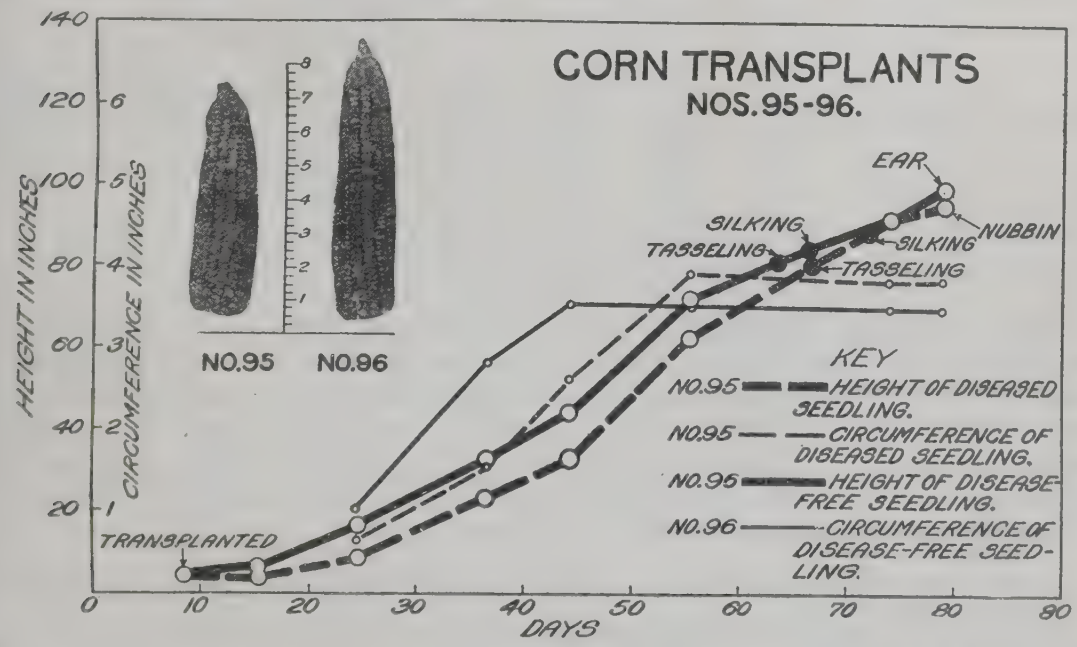

FIG. 3.—Growth curves and yield of diseased (No. 95) and healthy (No. 96) corn transplants. The greatest difference in height and stalk circumference occurred during the interval between 25 and 45 days after planting. After that time the diseased transplant seemed gradually to recover as far as size was concerned. This apparent recovery, however, was of no significance, for it produced only a short ear which was badly rotted, whereas the healthy transplant produced a sound ear of normal size.

TABLE I.—*Comparative field performance of 34 healthy and 34 diseased transplants of Funk Ninety-Day corn grown in clean soil, at Bloomington, Ill., in 1918*

Points considered.	Condition of seedlings when transplanted.	
	Apparently disease-free.	Diseased.
Number of seedlings:		
Transplanted	34	34
Dying within 16 days after transplanting	0	16
Living throughout season	34	18
Number of transplants producing:		
Two ears	7	1
One well-developed ear	18	4
One midsized ear	3	5
One nubbin only	3	4
Number of barren plants	3	4

CORRELATION OF EARLY HEIGHT AND YIELD OF FIELD-GROWN PLANTS

Table II gives the average heights, average yields, and correlation coefficients of height and yield of the plants in two contiguous plots of corn.

The plant measurements were taken 35 days, 56 days, and 146 days after planting.

TABLE II.—*Correlation of height (determined at different dates after planting) and yield, in Reid Yellow Dent grown from relatively disease-free and diseased seed in infested soil, at Bloomington, Ill., in 1918*

Condition of seed.	Number of plants.	Days after planting.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
			Cm.	*Gm.*	°	°
Relative disease-free	157	35	91. 1	201. 1	+0. 512	±0. 046
	157	56	187. 3	201. 1	+ .512	± .046
	157	146	281. 9	201. 1	+ .319	± .056
Diseased	119	35	86. 1	181. 2	+ .474	± .042
	119	56	189. 1	181. 2	+ .455	± .043
	119	146	275. 1	181. 2	+ .562	+ .037

The correlation coefficients in this table range from nearly 6 times to nearly 15 times the probable error. Love, of the Plant Breeding Department of Cornell University, and others, express the opinion that correlation coefficients which are more than 3.8 times the probable error are somewhat significant. King, as quoted by Babcock and Clausen (1), gives the following rules for the interpretation of the coefficient of correlation according to its relation to the probable error:

1. If the correlation coefficient is less than the probable error, there is no evidence whatever of correlation.
2. If the correlation coefficient is more than six times the size of the probable error, the existence of correlation is a practical certainty.
3. In cases where the probable error is relatively small:
   (a) If the correlation coefficient is less than 0.3 the correlation can not be considered at all marked.
   (b) If the correlation coefficient is above 0.5 there is a decided correlation.

Table III gives correlation coefficients between stalk circumference and yield of the same plants studied in Table II and presents the only correlations between circumference and yield which appear in this paper. They are given at this time in connection with the height data of the same plants to show that the two characters are seemingly well correlated.

TABLE III.—*Correlation of circumference of stalk (measured at different dates after planting) and yield in Reid Yellow Dent, grown from relatively disease-free and diseased seed on infested soil, at Bloomington, Ill., in 1918*

Condition of seed.	Number of plants.	Days after planting.	Average circumference.	Average yield per plant.	Correlation coefficient.	Probable error.
			Cm.	*Gm.*		
Relatively disease-free	157	35	5. 6	201. 1	+0. 618	±0. 038
	157	56	9. 4	201. 1	+ .478	± .088
	157	146	9. 0	201. 1	+ .643	± .036
Diseased	119	35	5. 3	181. 2	+ .643	± .036
	119	56	9. 8	181. 2	+ .568	± .030
	119	146	9. 2	181. 2	+ .620	± .033

TABLE IV.—*Correlation of early height (36 days after planting) and yield in Funk Ninety-Day corn grown on clean soil from relatively disease-free and diseased seed, at Bloomington, Ill., in 1919*

Condition of seed.	Number of plants.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
		Cm.	*Gm.*		
Disease-free	354	150.4	191.0	+0.359	±0.031
Diseased	331	146.3	179.4	+ .435	± .030
Seed selected from normally ripened plants	243	156.3	212.6	+ .305	± .039
Seed selected from prematurely ripened plants	244	154.0	197.0	+ .307	± .039

The experimental plots represented in Table IV were not planted until the latter part of the normal corn planting season. The soil in these plots had not grown corn during the previous 10 years. These facts probably account for the correlation coefficients being lower than those given in Table II. Yet it seems that, even under these conditions, the correlation between early height and yield is significant.

TABLE V.—*Correlation of height and yield in Bloody Butcher corn grown from artificially pollinated ears. Seed planted May 30 in infested soil at Bloomington, Ill., in 1919*

Group No.	Condition of parent plant.	Treatment of seed.	Number of plants.	Days after planting.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
					Cm.	*Gm.*		
I	Diseased plants selfed.	Uninoculated	265	30	67	132	+0.167	±0.040
		Inoculated	200	30	57	123	+ .360	± .041
II	Apparently disease-free plants crossed	Uninoculated	240	30	75	218	+ .405	± .037
		Inoculated	208	30	72	240	+ .366	± .045
		Uninoculated	240	52	213	218	+ .569	± .029
		Inoculated	208	52	211	240	+ .429	± .038
III	Apparently disease-free plants selfed.	Uninoculated	72	30	56	254	+ .432	± .064
		Inoculated	57	30	43	233	+ .537	± .063
		Uninoculated	72	52	210	254	+ .600	± .051
		Inoculated	57	52	199	233	+ .680	± .048
IV	Diseased plants crossed.	Uninoculated	65	30	92	155	+ .447	± .067
		Inoculated	50	30	93	113	+ .007	± .095
		Uninoculated	65	52	211	155	+ .650	± .048
		Inoculated	50	52	204	113	+ .205	± .085

In each group shown in Table V, series of plantings of seed inoculated with *Gibberella saubinetii* were made along with corresponding series with uninoculated seed.

There seems to be no special relation between the correlation coefficients and inoculation in the four different groups. However, there is in most cases a strong correlation between early height and yield. The correlation coefficients are higher for the measurements taken 52 days after planting than for those taken 30 days after planting. This can be accounted for by the fact that weak plants make slower gains in height during that period of their development (Tables VIII and IX). It will be noted that the inoculation (*Gibberella saubinetii*) materially reduced

the stand in every comparison and the average yield per plant in every one except group 2. In this group there was evidence of resistance, which probably accounts for the different response of the plants in this group to the inoculation.

RELATION OF EARLY VIGOR AND YIELD AS INFLUENCED BY SEED INFECTION

STUDIES OF INDIVIDUAL PLANTS

General observations, growth records, and yield data on the experimental plots planted with apparently disease-free and diseased seed impressed the authors with the fact that the recorded differences in stand did not accurately represent the actual differences between the various plots of corn in their early stages of growth. The classification of the plants as vigorous, semivigorous, or weak was an attempt to record more accurately the comparative condition of the plots at that time. Although this classification may be subject to personal error at times, it has been used advantageously in expressing significant differences in early vigor.

When this classification was first used a number of plants were selected for study about whose classification as vigorous, semivigorous, or weak, there was no doubt (Pl. 1 and 5). The plants were harvested individually and the dry-shelled weight of each ear determined. These data are presented in Table VI, where one may note the strong correlation existing between early vigor and yield, both in average production and in absence of barren stalks. Occasionally, however, plants classified as vigorous in the juvenile stage either may be barren or produce nubbins only; but this is the exception rather than the rule. At least one important disease of corn that does not affect early vigor appreciably appears to cause a certain amount of barrenness and nubbin production. This phase of the corn, root and stalk rot problem will be discussed in another paper.

TABLE VI.—*Relation of early vigor to yield and barrenness in plants of Reid Yellow Dent, classified as vigorous, semivigorous, or weak, 35 days after planting, at Bloomington, Ill., in 1918*

Points considered.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants	601	259	250
Percentage of plants dying	3.3	3.2	9.8
Average yield per plant (in grams)	249.4	139.9	100.5
Percentage of barren plants	6.5	32.8	50.0

Table VII gives data on an experiment conducted with disease-free and diseased seed of Funk Ninety-Day corn. The plot was divided and one-half was planted with each kind of seed. A glance at the percentage of vigorous and weak plants grown from relatively disease-free seed as compared with the plants grown from diseased seed shows that the percentage of vigorous plants drops from 64.3 in the disease-free half to 29.1 in the diseased half, while the percentage of weak plants rises from 8.8 to 19.1. Early vigor and average yield of grain per plant, as well as absence of barren stalks, are directly correlated.

TABLE VII.—*Relation of early vigor 30 days after planting to yield, as influenced by seed infection in Funk Ninety-Day corn, grown from relatively disease-free and diseased seed on infested soil, at Bloomington, Ill., in 1918*

Points considered.	Relatively disease-free seed.			Diseased seed.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants..	139	58	19	55	98	36
Percentage of plants in each group.....	64. 3	26. 9	8. 8	29. 1	51. 8	19. 1
Average yield per plant (in grams).....	243. 5	207. 2	97. 7	241. 5	172. 7	86. 8
Percentage of barren plants.....	. 1	3. 5	26. 3	0	7. 2	50. 0

TABLE VIII.—*Relation of early vigor to rate of growth and yield as influenced by seed infection in Funk Ninety-Day corn grown on clean soil at Bloomington, Ill., in 1919*

Points considered.	Relatively disease-free seed.			Slightly diseased seed.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants..	146	159	45	123	165	38
Percentage of plants in each group.....	41. 8	45. 6	12. 8	37. 7	50. 6	11. 7
Average height (in centimeters) 15 days after planting.....	44. 3	37. 7	26. 8	43. 3	36. 2	24. 4
Average height (in centimeters) 36 days after planting.....	158. 0	146. 6	123. 8	158. 8	143. 4	114. 5
Average increase in height per plant (in centimeters) 21 days after first record.....	113. 7	108. 9	97. 0	115. 5	107. 2	90. 1
Average yield per plant (in grams).....	199. 9	192. 0	158. 0	184. 6	176. 6	135. 8

TABLE IX.—*Relation of early vigor to rate of growth and yield of Funk's Ninety-Da corn as influenced by pathologic condition of plants from which seed was selected, grow on clean soil at Bloomington, Ill., in 1919*

Points considered.	Seed from ears ripening normally.			Seed from ears ripening prematurely.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants	78	138	27	61	156	27
Percentage of plants in each group	32. 1	56. 8	11. 1	25. 0	63. 9	11. 1
Average height (in centimeters) 15 days after planting	45. 7	38. 5	27. 4	44. 4	36. 8	28. 8
Average height (in centimeters) 36 days after planting	169. 0	154. 2	126. 6	170. 1	150. 3	130. 4
Average increase in height per plant (in centimeters) 21 days after first record	123. 3	115. 7	99. 2	125. 7	114. 5	101. 6
Average yield per plant (in grams)	220. 5	215. 4	177. 8	220. 2	194. 8	147. 1

Tables VIII and IX are of special interest because of the fact that the classification of plants as vigorous, semivigorous, and weak was made only 15 days after the corn was planted and because the corn was planted at the end of the normal corn planting season in soil that had not grown corn for 12 years. These results lend further emphasis to the strong correlation of early vigor and yield.

In the experiments reported in Tables VIII and IX, the heights of the plants were taken 15 days after planting at the time of judging vigor, and again at 36 days after planting. In both measurements there is a fairly wide variance in the average heights of vigorous, semivigorous, and weak plants. In the period of 21 days between the first and second measurements all groups of plants made considerable gains in heights. A comparison of the average gains in plant height during this period shows that, in every comparison, the vigorous plants made the greatest gains, followed by the semivigorous plants, and then by the weak. It is evident that the more vigorous individuals not only retain their initial superiority in height but grow more rapidly than the less vigorous plants. The difference between the vigorous and weak plants in this respect is very marked.

STUDIES OF PLANT POPULATIONS

From the foregoing data and discussion it seems evident (1) that early vigor and yield of grain are closely correlated (Pl. 4), also (2) that the percentages of strong, vigorous plants in populations grown from diseased seed are lower than in similar populations grown from relatively disease-free seed (Pl. 6 and 7). Inasmuch as the total yield of a plot or field of maize depends on the total number of plants and the average yield per plant, it follows that the total yields of contiguous plots, differing from 0 to 8 per cent in field stand, will be more or less directly proportional to the percentages of strong, vigorous plants in the populations. A large number of experiments have been conducted during a

period of four years in which alternate plots were planted with relatively disease-free and diseased seed. Where field stands and other factors were reasonably comparable, there has been a reduction in the percentage of vigorous plants, as classified 25 to 35 days after planting, as well as a reduction in final acre yield in bushels, although the relation varied from year to year, depending on seasonal conditions. These data are summarized in Table 10. The 1920 data are presented in more detail and discussed later in this paper. A part of the 1921 data is presented in both detailed and summarized forms to show more clearly the methods used in the investigations on which this paper is based.

TABLE X.—*Summary of data showing influence of seed infection on early vigor and yield of corn grown from relatively disease-free and diseased seed, at Bloomington, Ill., in 1918, 1919, 1920, and 1921*

Year.	Number of experiments.	Total number of plants classified.	Average reduction in percentage of vigorous plants.	Average increase in percentage of weak plants.	Average decrease in acre yield.
					Bushels.
1918	7	22,479	9.7	4.8	14.4
1919	7	29,013	13.3	6.3	12.9
1920	8	9,318	15.4	4.2	8.6
1921	4	6,134	22.6	4.9	9.2

RELATION OF EARLY VIGOR AND YIELD AS INFLUENCED BY PATHOLOGIC CONDITION OF MOTHER PLANT

In the fall of 1917, attractive ears were selected from vigorous and apparently healthy plants and from plants whose roots and stalks were rotted. The seed was planted the following spring in ear rows, the seed from apparently healthy plants being alternated with the seed from diseased plants. Similiar experiments were conducted during the following two years. The data from these experiments are given in Table XI. Seed ears selected from plants whose roots and stems are badly rotted may or may not be infected with the rot pathogenes. In many cases they are not infected to such an extent that the infection can be detected by a careful germination test. But when these ears are used for seed they usually produce plants that are very susceptible to the root and stalk rots.

TABLE XI.—*Early vigor and yield as influenced by the pathologic condition of mother plants from which seed was selected. The table shows reduced vigor and reduced yield in plants grown from seed selected from diseased plants, at Bloomington, Ill.*

Year.	Variety.	Number of plants.	Reduction in stand.	Reduction in vigorous plants.	Increase in weak plants.	Decrease in acre yield.	Reduction in yield.
			Per cent.	*Per cent.*	*Per cent.*	*Bushels.*	*Per cent.*
1918	Reid Yellow Dent	5,140	10.9	4.4	1.2	7.3	10.9
1918	Funk 90-Day	6,617	.5	14.1	2.8	11.4	16.3
1919	do	2,090	2.1	15.4	1.3	6.8	9.2
1919	do	927	1.5	5.5	1.6	2.5	4.3
1920	Reid Yellow Dent	1,357	5.2	9.5	1.0	12.4	16.5
1920	do	3,144	.1	4.7	1.2	6.0	7.5

A glance at Table XI shows that seed selected from diseased mothe plants produces plants deficient in early vigor and lacking in the ability to produce high grain yields. This is especially pronounced when the seed is planted in infested soil.

Seed ears from badly diseased plants usually are lacking in luster, and physical examination of the kernels on such ears frequently shows them to be more or less starchy (*21*). Specific gravity determinations on these kernels also reveal a tendency toward decreased density. Table XII gives further data regarding the comparative field performance of starchy and slightly starchy seed, as compared with the harder, more horny seed. The field stands and seed infections were comparable in all cases. Ordinarily, however, as Trost and Hoffer (*38*) have reported, ears with starchy kernels are very often infected with one or more of the corn rot pathogenes.

TABLE XII.—*Influence of endosperm composition (physical determination) on early vigor and yield. Data show reduced vigor and reduced yield in plats planted with starchy and slightly starchy seed as compared with similar plots grown from horny seed at Bloomington, Ill.*

Year.	Condition of seed.	Total number of plants.	Reduction in vigorous plants.	Increase in weak plants.	Reduction in acre yield.	Reduction in acre yield.
			Per cent.	*Per cent.*	*Bushels.*	*Per cent.*
1918	Starchy	1,273	14.3	2.7	13.5	19.7
1918	Slightly starchy	9,243	7.7	3.0	9.9	14.5
1919	Starchy (individual plants)	170	14.0	4.0	.....	8.1
1920	Slightly starchy	926	21.7	1.0	4.8	9.0

RELATION OF EARLY VIGOR TO SIZE OF EARS PRODUCED

As might be expected from the data presented up to this point, early vigor and size of ears are strongly correlated. Plants vigorous and apparently healthy in their early stages of growth are much more likely to produce good-sized and well-matured ears than plants stunted during this same period. This fact is brought out clearly in Table XIII.

TABLE XIII.—*Relation of early vigor to the size of ears on 1,715 individual corn plants grown at Bloomington, Ill. in 1918*

Variety and condition of seed.	Percentage of plants classified in their early stages of growth as vigorous, semivigorous, and weak which produced good-sized, well-matured ears.		
	Vigorous.	Semivigorous.	Weak.
Reid Yellow Dent	67.5	18.9	15.1
Funk Ninety-Day (relatively disease-free)	92.0	53.4	0
Funk Ninety-Day (diseased seed)	83.6	54.1	2.0

RELATION OF EARLY VIGOR TO SHRINKAGE OF GRAIN

It has been found, as East and Hayes (*11*) and Grantham (*14*) have reported, and as many other investigators have observed, that weak plants are from 7 to 10 days later in tasseling and silking than vigorous

plants. Observations and records on many hundreds of individual plants at frequent intervals over a period of four years have indicated that plants stunted in their early growth as a result of attacks by the root and stalk rot pathogenes are nearly always somewhat later in tasseling and silking (figures 1, 2, and 3). These plants, therefore, on account of delayed pollination and fertilization combined with their weakened condi-

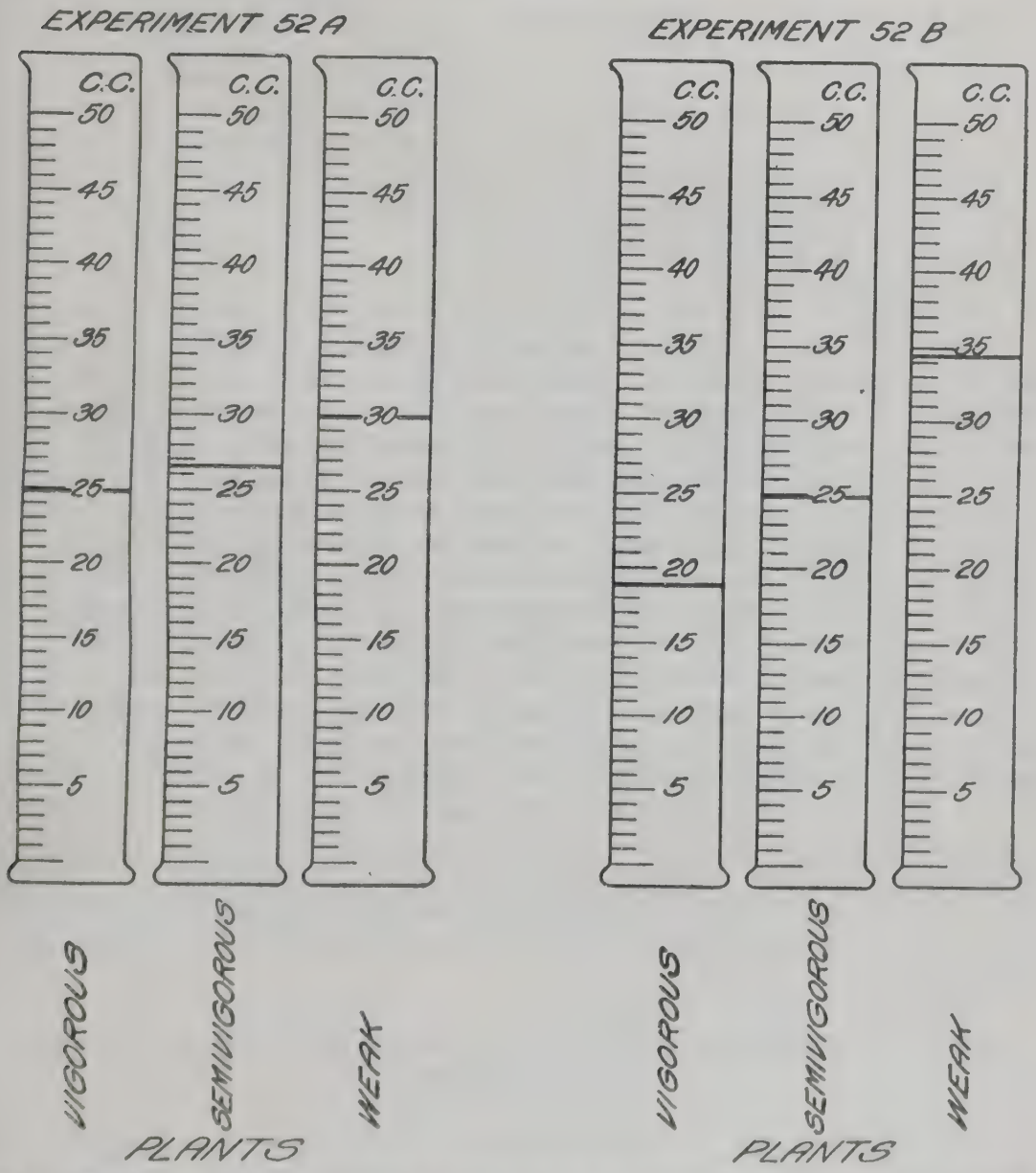

FIG. 4.—Graph showing cubic centimeters of water lost in the curing of 100 gm. of freshly harvested corn produced on vigorous, semivigorous, and weak plants, grown from uninoculated seed. (See Table XIV.)

tion, can not mature their grain as early nor as completely as the more nearly normal plants. As a result, the corn from the weak plants and from diseased plants usually contains more moisture when harvested and consequently shrinks more when reduced to an air-dry condition. Some data on this are shown in Table XIV.

TABLE XIV.—*Relation of early vigor to shrinkage in weight of grain, determined on 1,325 plants of Funk's Ninety-Day corn in two inoculation experiments, alternate hills of which were inoculated at planting time with Gibberella saubinetii grown on clean soil of high fertility at Bloomington, Ill., in 1919*

Experiment No.	Treatment of seed.	Shrinkage of grain from plants classified in their early growth stages as vigorous, semivigorous, or weak.		
		Vigorous.	Semivigorous.	Weak.
		Per cent.	*Per cent.*	*Per cent.*
52 A	Uninoculated	24.7	26.4	30.1
	Inoculated	26.4	27.3	27.2
52 B	Uninoculated	18.9	24.8	34.4
	Inoculated	21.5	28.4	29.2

Table XIV gives the percentage of shrinkage of the grain from vigorous, semivigorous, and weak plants harvested only a few days before a killing frost. The product from each plant was weighed separately when harvested and again after the ears were thoroughly air-dried. The results show that in every case there is a greater percentage of shrinkage in the grain produced by the weak than by the vigorous plants (fig. 4). Plants of medium vigor tend to have an intermediate percentage of shrinkage. This indicates that the same factors which enable the more vigorous plants to grow most rapidly and to excel in grain production also cause the grain to mature earlier. At harvest time there was little apparent difference in the height of plants that were classified in their early stages of growth as vigorous, semivigorous, and weak. Incidentally it is of interest to note that there is a slightly greater shrinkage in the product of the inoculated rows than in that of the uninoculated in the vigorous and semivigorous groups. In the groups of weak plants the situation is reversed, the weak plants having a smaller percentage of shrinkage in the inoculated than in the uninoculated rows. This may be accounted for by the fact that many of the weak plants in the inoculated rows died prematurely, thus permitting partial curing before harvest.

RELATION OF EARLY VIGOR AND QUALITY OF PRODUCT AS INFLUENCED BY SEED INFECTION

DATA OBTAINED IN 1920

In addition to the reduced size of ear and reduced yield of shelled grain from plants semivigorous or weak in early vigor as compared with the yield of plants strong and apparently healthy in their early stages of growth, there is a distinct difference in the quality of the grain. Because many of the less vigorous and diseased plants are killed by frost before the grain is mature, there are more rotted ears and more light chaffy ears in the corn produced from semivigorous and weak plants than from vigorous plants under comparable conditions. As brought out in previous tables, corn grown from diseased seed contains fewer strong plants and more weak plants than corn grown from relatively disease-free seed. Consequently, it would be expected that corn grown from diseased seed would contain more nubbins, more rotted ears, and more light, chaffy ears

than corn grown from nearly disease-free seed. Much of this corn of inferior quality would be left in the field unharvested by most farmers. Many farmers who might gather such corn from the field probably would either sort it out when dumping the corn at the crib or when shelling it for market. At any rate, small nubbins, rotted ears, and chaffy ears represent corn of low grade and low selling value.

In view of these facts it was deemed advisable, beginning in 1920, to report separate yields of marketable and unmarketable corn. Plates 2, 3, and 4 illustrate the kinds of corn that were classified as marketable and unmarketable. There are occasional ears about whose classification there might be some question; but, on the whole, personal judgment changes but little within a given experiment, and errors due to personal factors are comparatively few when a large number of experiments are concerned. Although this procedure is imperfect, it gives a more accurate index to the value of any yield of corn than total yield alone. When considering yield under actual farm conditions, quality should be as important as quantity, a fact forcibly emphasized in the 1921 data.

In presenting data from experiments conducted in 1920 and 1921, it seemed desirable to report yields in terms of percentages of increased yield of nearly disease-free over the moderately diseased seed, inasmuch as the moderately diseased seed used was representative of the majority of seed ears used by the farmers supplying the seed for the experiments. The yields from these different lots of moderately diseased seed were higher than the average corn yield of the counties in which the experiments were conducted. On the Agronomy South Farm of the Illinois Agricultural Experiment Station at Urbana, these lots of moderately diseased seed compared favorably in yield with the standard varieties. The nearly disease-free seed lots used in the experiments (1920 and 1921) were practically free from any of the root and stalk rot pathogenes, as evidenced by repeated laboratory determinations, and represented the best seed ears available in the open fertilized seed stocks of the Corn Belt.

TABLE XV.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn. Reid Yellow Dent (Macon County strain) grown on infested soil of medium fertility, at Bloomington, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	*Per cent.*
Number of plants in experiment	727	846	.....	.....
Early vigor:				
Percentage of kernels producing plants	75.8	88.1	16.2	.....
Percentage of kernels producing vigorous plants	29.2	45.3	55.2	.....
Percentage of kernels producing weak plants	12.3	6.3	.....	48.8
Yield:				
Total acre yield (in bushels)	49.8	63.5	27.5	.....
Acre yield of marketable corn	33.7	54.6	62.0	.....
Percentage of nubbins (by count)	23.3	11.6	.....	50.2
Percentage of rotted ears (by count)	10.7	3.9	.....	63.5
Percentage of chaffy ears (by count)	26.0	7.7	.....	70.4

Table XV gives data showing the influence of seed infection on early vigor and subsequent yield and quality. The increased percentage of

strong, vigorous plants in the plots planted with nearly disease-free seed is very marked (Pl. 6), as is the decrease in percentage of nubbins, rotted ears, and chaffy ears. The increase in yield of marketable corn is very significant. The relation of increased early vigor to increased yield of marketable corn is brought out more clearly in figure 5.

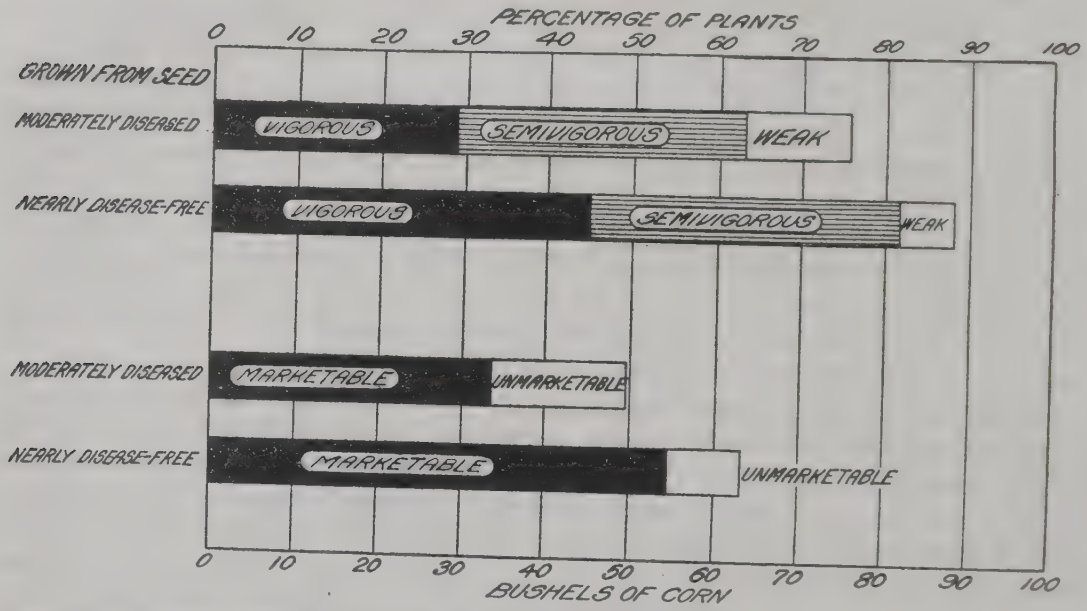

FIG. 5.—Graphic presentation of Table XV, showing relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

TABLE XVI.—*Influence of moderately diseased and nearly disease-free seed of Funk strain of Reid Yellow Dent, on early vigor and yield of marketable corn, when grown on infested soil of medium fertility, at Bloomington, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
Number of plants in experiment	323	361	*Per cent.*	*Per cent.* .....
Early vigor:				
Percentage of kernels producing plants	84.1	91.4	8.6	.....
Percentage of kernels producing vigorous plants	23.4	34.4	47.0	.....
Percentage of kernels producing weak plants	8.3	2.6	.....	68.7
Yield:				
Total acre yield (in bushels)	57.3	61.7	7.6	.....
Acre yield of marketable corn (in bushels)	42.1	54.7	29.9	.....
Percentage of nubbins (by count)	11.8	8.2	.....	30.5
Percentage of rotted ears (by count)	4.3	3.6	.....	19.4
Percentage of chaffy ears (by count)	12.5	2.6	.....	79.2

Table XVI gives further data on the influence of seed infection on early vigor and subsequent yield. Figure 6 shows graphically the data given in Table XVI.

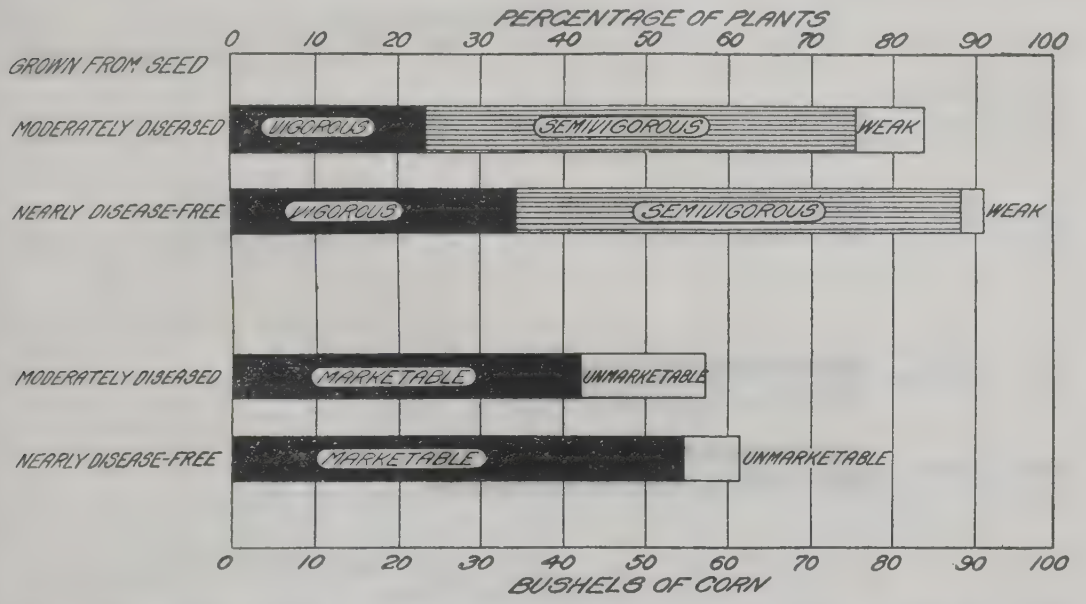

FIG. 6.—Graphic presentation of Table XVI, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

TABLE XVII.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of McKieghan strain of Reid Yellow Dent on soil of average fertility on which clover had been grown for the previous two years but which had previously never been limed nor phosphated (22), at Yates City, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	*Per cent.*
Number of plants in experiment	1,082	1,251	.....	.....
Early vigor:				
Percentage of kernels producing plants	75.1	86.8	15.6	.....
Percentage of kernels producing vigorous plants	29.4	39.1	33.0	.....
Percentage of kernels producing weak plants	16.2	13.1	.....	19.1
Yield:				
Total acre yield (in bushels)	67.0	72.4	8.1	.....
Acre yield of marketable corn (in bushels)	44.5	52.4	17.8	.....

The increase in yield of marketable corn shown in Table XVII and figure 7 is not so marked as in previous tables, but it is large enough to be significant.

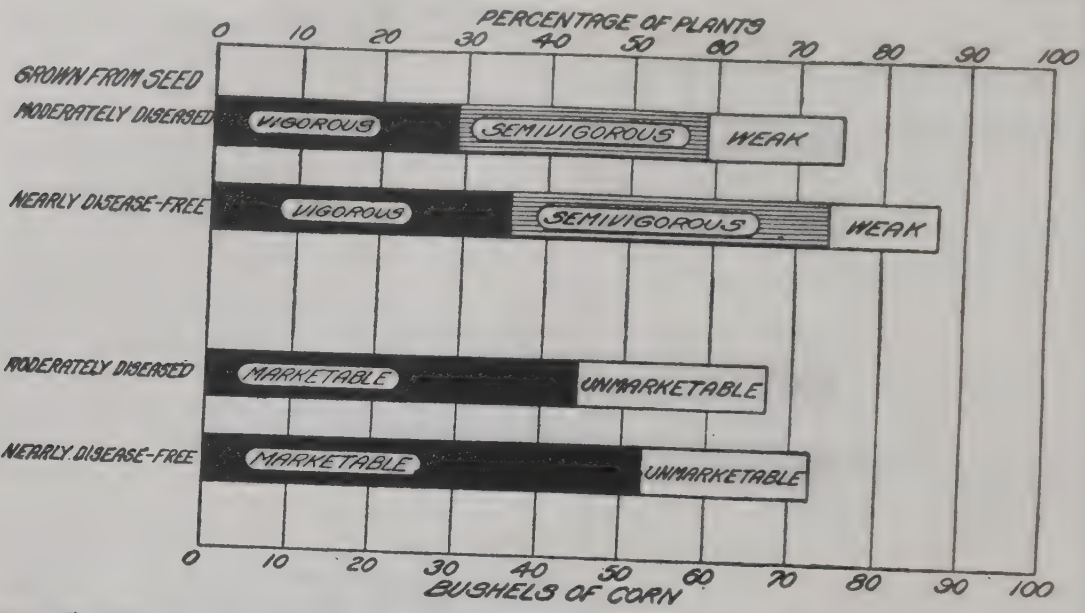

FIG. 7.—Graphic presentation of Table XVII, showing relation of diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

Tables XVIII, XIX, and XX give the results of similar experiments conducted on the Agronomy South Farm of the Illinois Agricultural Station at Urbana, Ill., during 1920, on very fertile soil (fig. 8), on soil of good average fertility, and on soil which has had 75 per cent of corn in the rotation for 13 years.

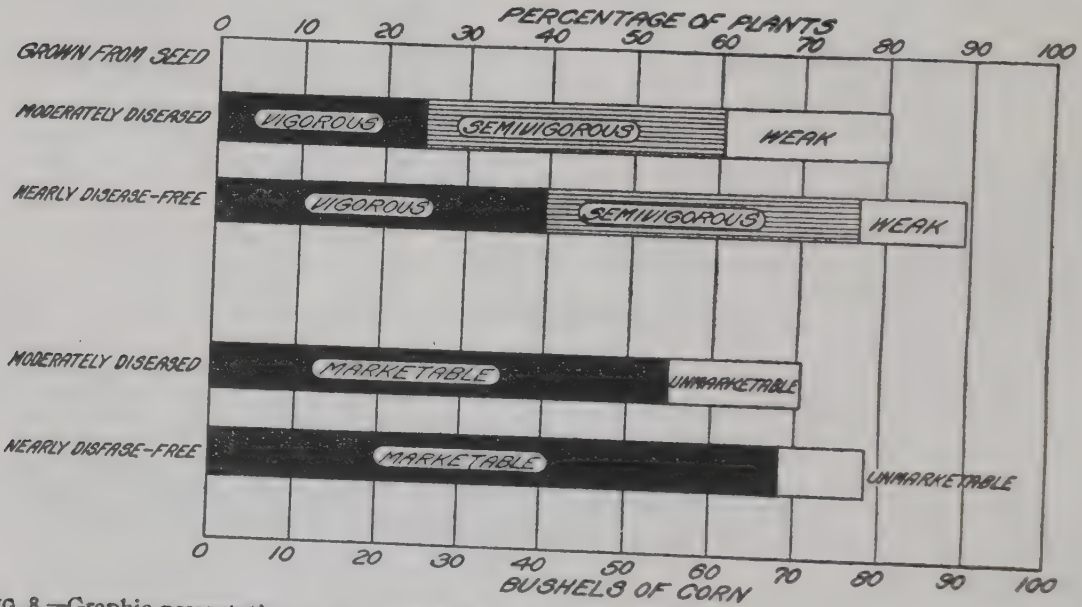

FIG. 8.—Graphic presentation of Table XVIII, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn, in bushels per acre.

In every case there has been a marked increase in the field stand, percentage of strong, vigorous plants, and yield of marketable corn in the plots grown from nearly disease-free seeed. In Table XVIII the difference in stand might account for the difference in total yield, but the difference in yield of marketable corn must be accounted for in some other way. Figure 8 shows the relation of the increase in percentage of vigorous plants to the increase in yield of sound, marketable corn.

TABLE XVIII.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on very fertile soil to which rock phosphate and manure has been applied, at Urbana, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	*Per cent.*
Number of plants in experiment	3,850	4,281	.....	.....
Early vigor:				
Percentage of kernels producing plants	80.3	89.3	11.2	.....
Percentage of kernels producing vigorous plants	25.2	39.4	56.3	.....
Percentage of kernels producing weak plants	19.5	12.3	.....	36.9
Yield:				
Total acre yield (in bushels)	70.7	78.7	11.3	.....
Acre yield marketable corn (in bushels)	54.8	68.2	24.5	.....
Percentage of nubbins (by count)	19.5	14.1	.....	27.7
Percentage of rotted ears (by count)	4.5	3.3	.....	26.7
Percentage of chaffy ears (by count)	8.5	4.3	.....	49.4

The same standard for vigorous plants was maintained in taking the data reported in Tables XVIII, XIX, and XX. In the experiments reported in Table XX there was little apparent difference in vigor or general appearance in the early stages of growth between the rows grown from diseased and nearly disease-free seed. However, it was evident upon close examination that the plants in plots planted with nearly disease-free seed were somewhat larger in circumference at the base and also sturdier. The increase in yield from the better seed is very marked, even in heavily infested soil. Such results suggest that the nearly disease-free seed may have possessed some degree of resistance to the root and stalk rots.

TABLE XIX.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on soil of average fertility, limed and phosphated, at Urbana, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	*Per cent.*
Number of plants in experiment	3,847	4,263	.....	.....
Early vigor:				
Percentage of kernels producing plants	80.2	88.8	10.7	.....
Percentage of kernels producing vigorous plants	9.6	19.1	99.0	.....
Percentage of kernels producing weak plants	30.5	22.9	.....	24.9
Yield				
Total acre yield (in bushels)	67.7	79.4	17.3	.....
Acre yield of marketable corn (in bushels)	59.6	74.1	24.3	.....

TABLE XX.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on heavily infested soil, limed and phosphated, at Urbana, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	*Per cent.*
Number of plants in experiment	2,096	2,338	.....	.....
Early vigor:				
Percentage of kernels producing plants	74.5	83.4	11.9	.....
Percentage of kernels producing vigorous plants	1.7	2.6	52.9	.....
Percentage of kernels producing weak plants	59.3	59.2	.....	0.2
Yield:				
Total acre yield (in bushels)	39.2	47.9	22.2	.....
Acre yield of marketable corn (in bushels)	32.4	41.3	27.5	.....

DATA OBTAINED IN 1921

The season of 1921 was different in many respects from the seasons of the previous three years. As a result of fungus injury following the unusually heavy attacks of the corn earworm (*Chloridea obsoleta* Fab.) during the warm, moist weather of the late summer and early fall, there was a very high percentage of rotted corn throughout the Corn Belt. In view of these facts the 1921 data relative to marketable and unmarketable corn in the yields from moderately diseased and nearly disease-free seed are of extraordinary significance. These data not only give further evidence on the important relations existing between seed infection, reduced early vigor, and reduced yields of sound corn, but show clearly that corn grown from seed infected with the root and stalk rot pathogenes, or seed susceptible to the root and stalk rots, is likely to be much more susceptible to fungi causing ear rots than corn grown from seed relatively free from these pathogenes. In most cases the different lots of corn were infested to approximately the same extent with earworms, but there was a vast difference in the degree to which the different lots were injured by the fungi following the attack of earworms. The principal fungi concerned with ear rots were *Diplodia zeae* and *Fusarium* spp.

The data presented in Tables XXI to XXV were secured from plantings on the no-treatment plots in a series of fertilizer experiments. These soil plots were not all equally fertile, but the soil variation within each plot probably was very slight. Consequently, comparisons should be made between the moderately diseased and nearly disease-free on each plot. The plantings on the various no-treatment plots were replications in which the same two seed composites were used throughout the four times of planting. The results are reported in Tables XXI to XXV and graphically presented in figures 9 to 13.

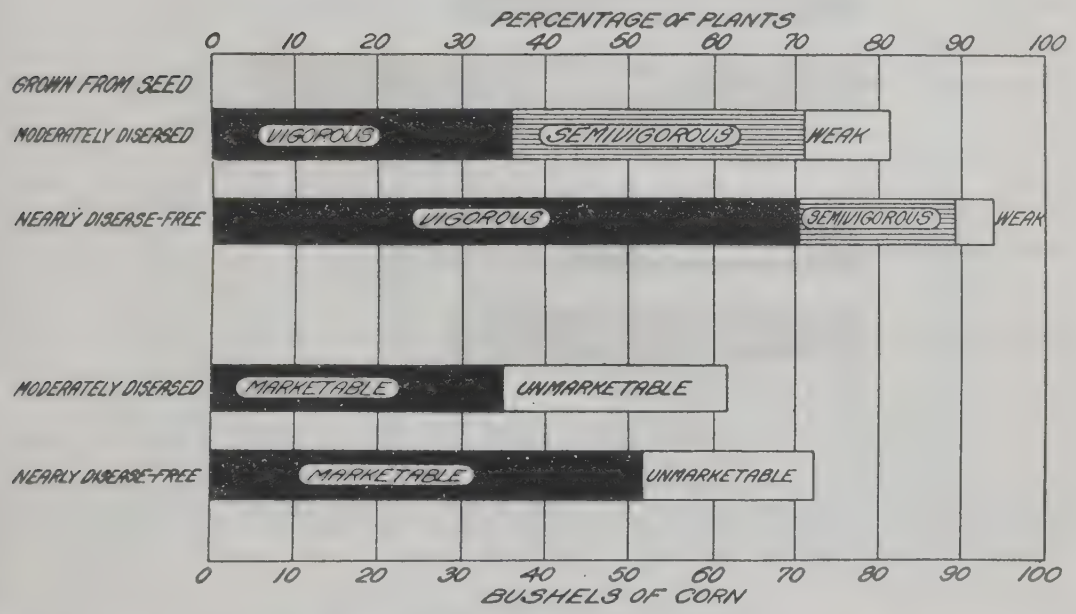

FIG. 9.—Graphic presentation of Table XXI, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

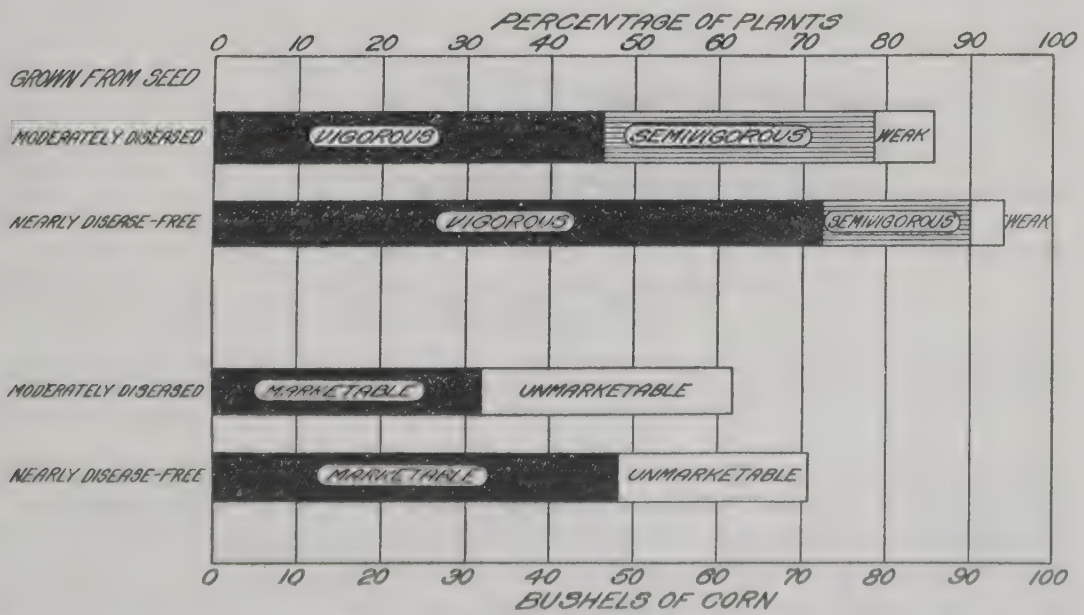

FIG. 10.—Graphic presentation of Table XXII, showing the relation of moderately diseased and nearly disease free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

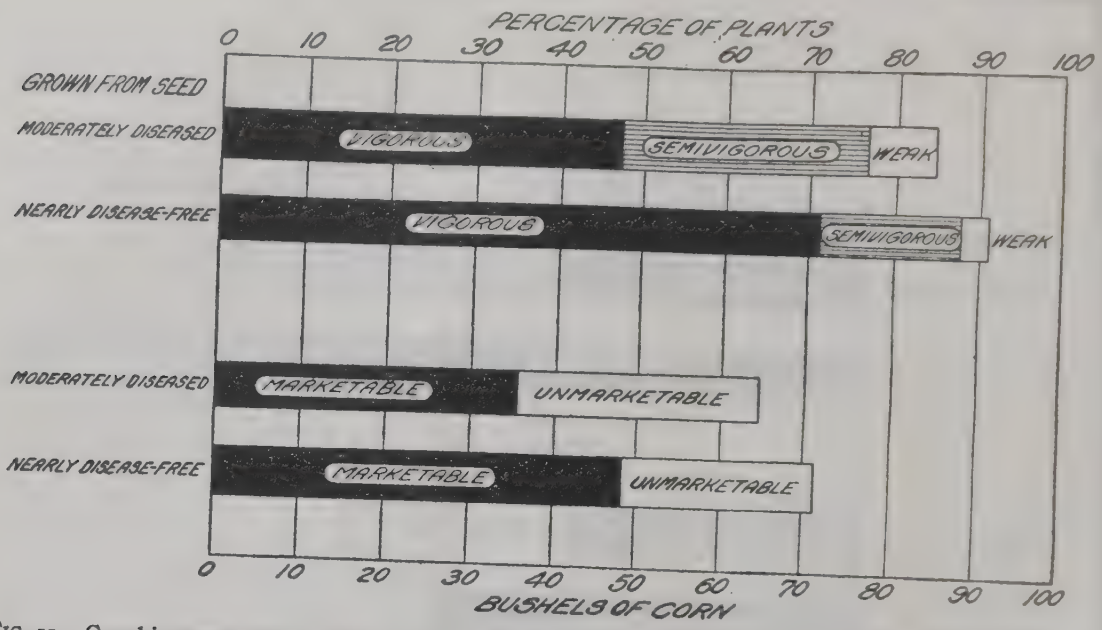

FIG. 11.—Graphic presentation of Table XXIII, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

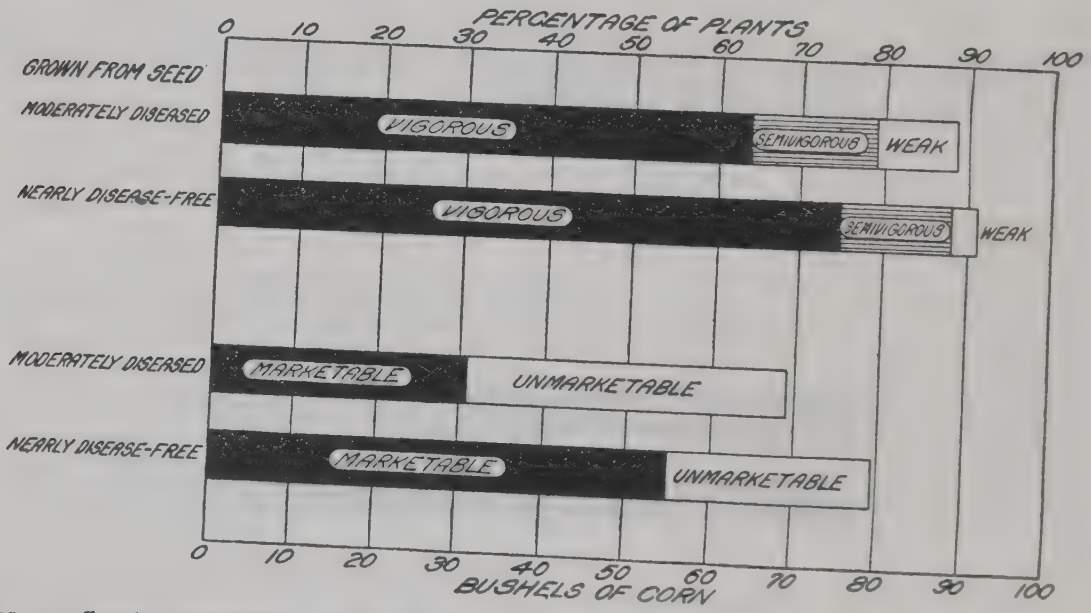

FIG. 12.—Graphic presentation of Table XXIV, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

The standard for vigorous plants in 1921 was somewhat different from that used during the previous three years of the investigations. On this account percentages of vigorous plants in 1920 can not be compared with percentages of vigorous plants in similar composites in 1921. However, the standards for vigorous, semivigorous, and weak plants were reasonably constant throughout each season, especially within any given experiment.

The soil was cold at the time the first planting was made and continued rather cold for 8 to 10 days. In this connection it is interesting to note that the field stand of the corn grown from moderately diseased seed is 12.2 per cent lower than that grown from the nearly disease-free composite (see Table XXI). By referring to Table XXV and figure 14 it

may be observed that the first planting showed the greatest difference in field stand between the moderately diseased and nearly disease-free composites.

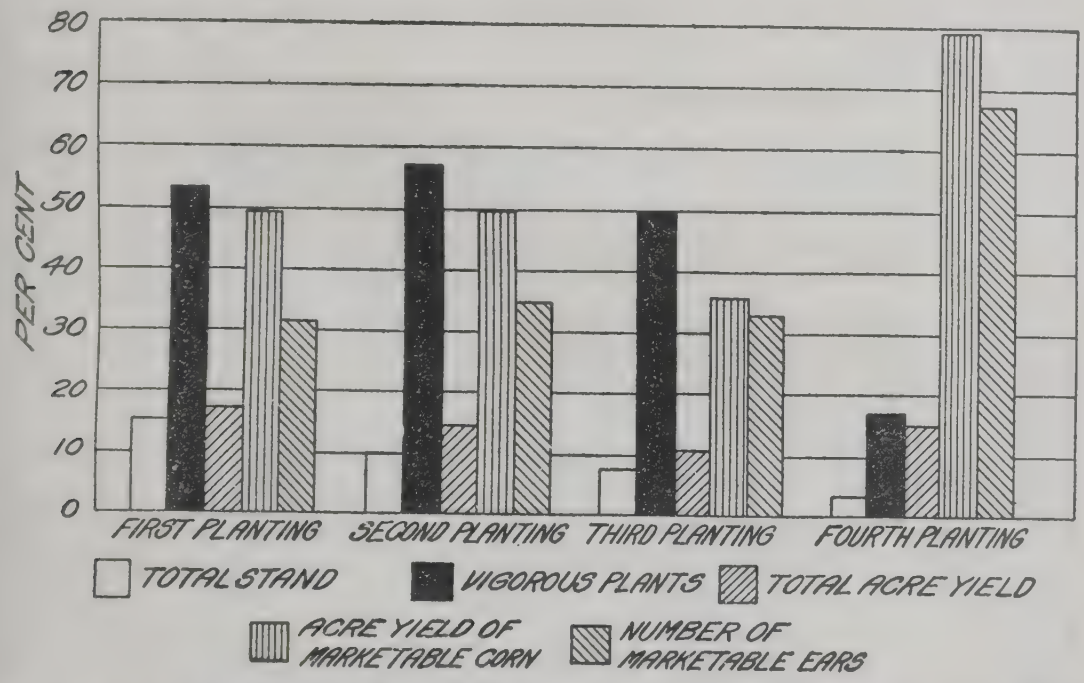

FIG. 13.—Graphic presentation of Table XXV, showing percentage increases in total stand, vigorous plants, total acre yield, acre yield of marketable corn, and number of marketable ears in favor of nearly disease-free seed over moderately diseased seed.

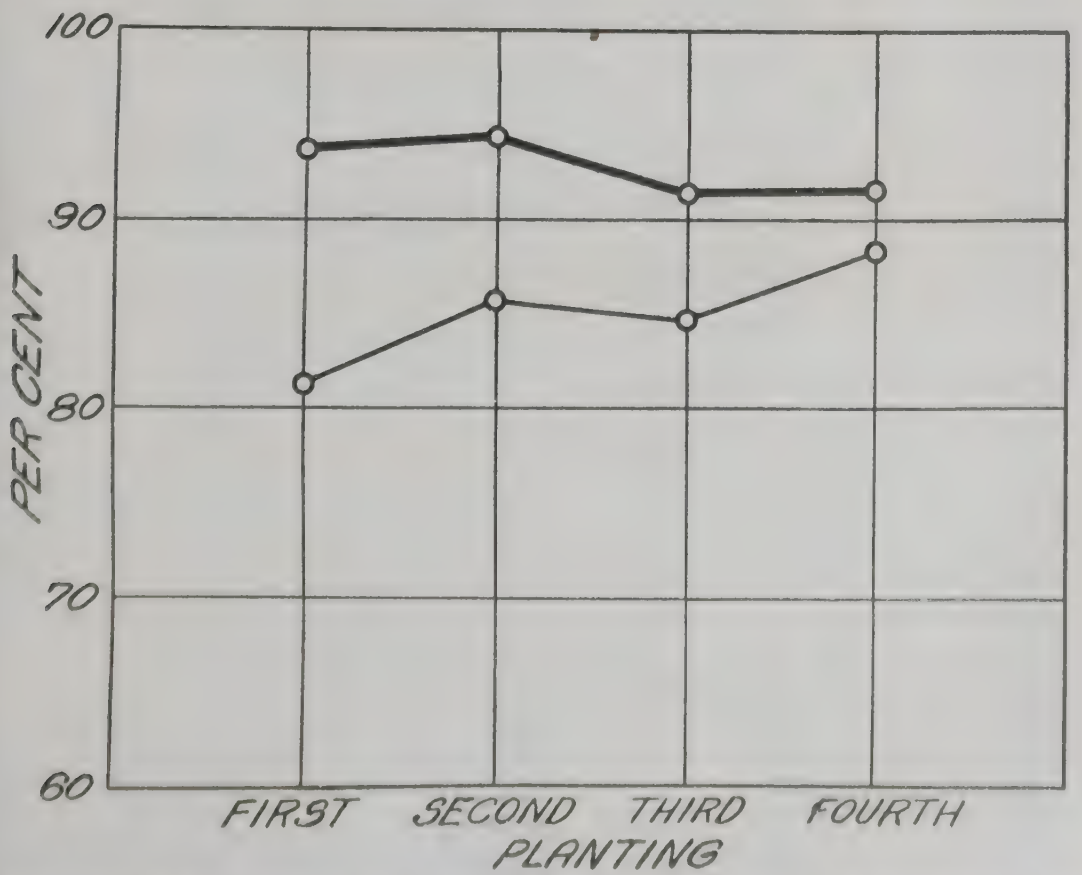

FIG. 14.—Graphic presentation of the percentage of field stand produced by the two lots of seed when planted at different dates, the heavy line representing nearly disease-free seed, the light line the moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

TABLE XXI.—*A comprehensive study of the principal data showing the influence of moderately diseased [a] and nearly disease-free [b] seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. First planting (May 7) of the same series shown in Tables XXII, XXIII, and XXIV. Bloomington, Ill., 1921*

Soil plot No.	Condition of seed.	Early vigor data.						Yield data.																					
		Total stand.		Vigorous plants.		Weak plants.						Marketable corn.						Unmarketable corn.				Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling percentage.	Moisture percentage.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Moisture percentage.	Weight of dry matter.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Lbs.	*Lbs.*	*P. ct.*	*Bus.*	*Bus.*	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*		*Lbs.*								
1	Moderately diseased.	81	84.4	48	50.0	14	14.6	48.5	41.2	15.0	71.0	36.2	22.0	18.8	85.4	16.6	15.7	19.2	17.8	15.4	15.1	32	50.8	15	23.8	15	23.8	1	1.6
	Nearly disease-free...	88	91.6	51	53.1	9	9.4	51.2	44.1	13.8	76.6	58.8	35.1	30.0	85.5	15.0	25.5	9.0	7.3	15.0	6.2	54	71.1	14	18.4	2	2.6	6	7.9
4	Moderately diseased.	80	83.3	39	40.6	17	17.7	45.7	37.4	18.1	63.5	23.8	13.7	12.0	87.6	14.1	10.3	23.7	20.2	14.7	17.2	20	29.0	27	39.1	14	20.3	8	11.6
	Nearly disease-free...	91	94.8	82	85.4	2	2.1	54.7	45.9	16.1	77.1	53.1	31.3	27.0	86.3	15.0	23.0	14.6	12.2	14.5	10.4	44	55.0	23	28.7	11	13.8	2	2.5
7	Moderately diseased.	81	84.4	50	52.1	11	11.5	45.6	37.1	18.6	62.6	38.8	22.9	19.8	86.5	15.0	16.8	14.2	12.1	14.8	10.3	35	51.5	16	23.5	11	16.2	6	8.8
	Nearly disease-free...	87	90.6	65	67.7	10	10.4	51.0	42.2	17.3	71.3	44.5	26.3	22.5	85.6	14.4	19.3	15.9	13.5	14.2	11.6	41	53.3	25	32.4	7	9.1	4	5.2
9	Moderately diseased.	75	78.1	44	45.8	9	9.4	43.9	34.9	21.0	59.1	33.7	19.8	17.0	85.8	14.2	14.6	15.1	12.8	14.2	11.0	27	46.5	13	22.4	10	17.3	8	13.8
	Nearly disease-free...	90	93.8	74	77.1	1	1.0	50.1	42.1	15.9	71.0	51.2	30.1	26.1	86.7	15.0	22.2	12.0	10.0	13.8	8.6	53	66.3	13	16.2	10	12.5	4	5.0
10	Moderately diseased.	83	86.4	51	53.1	8	8.3	47.0	37.8	19.6	62.6	37.4	22.3	19.2	86.1	15.6	16.2	15.5	12.8	15.2	10.9	33	45.2	18	24.6	14	19.2	8	11.0
	Nearly disease-free...	93	96.9	75	78.1	3	3.1	55.7	46.9	15.8	77.6	61.2	36.6	31.4	85.8	15.5	26.5	10.3	8.5	15.1	7.1	59	66.3	22	24.7	4	4.5	4	4.5
13	Moderately diseased.	71	74.0	41	42.7	8	8.3	42.3	34.4	18.7	56.7	33.9	20.3	17.5	86.2	16.1	14.7	14.1	11.7	15.3	9.9	29	49.1	16	27.1	7	11.9	7	11.9
	Nearly disease-free...	91	94.8	71	74.0	0	0.	57.6	48.9	15.1	81.2	56.3	33.7	29.0	86.1	16.0	24.4	15.2	12.8	15.8	10.8	55	61.8	13	14.6	14	15.7	7	7.9
16	Moderately diseased.	72	75.0	40	41.6	9	9.4	42.5	35.3	16.9	57.2	42.7	26.0	22.3	85.7	17.1	18.5	9.3	7.5	16.4	6.3	38	55.1	13	18.8	16	23.2	2	2.9
	Nearly disease-free...	92	95.8	68	70.8	7	7.3	49.5	40.2	18.7	66.6	46.8	28.2	23.8	84.4	14.7	20.3	12.0	10.0	14.0	8.6	49	55.7	18	20.4	10	11.4	11	12.5
19	Moderately diseased.	82	85.4	49	51.0	4	4.2	46.0	35.7	22.4	57.9	25.8	16.0	13.5	84.4	16.7	11.2	19.7	16.4	15.3	13.9	25	32.0	37	47.5	7	8.9	9	11.6
	Nearly disease-free...	88	91.6	58	60.4	4	4.2	49.2	39.4	19.9	65.1	39.9	23.9	20.4	85.4	16.6	17.0	15.5	13.0	15.9	10.9	42	51.9	24	29.6	9	11.1	6	7.4
21	Moderately diseased.	78	81.3	33	34.4	9	9.4	46.6	37.3	19.9	62.5	38.5	23.0	19.7	85.9	14.9	16.7	14.3	12.2	14.5	10.4	38	54.3	14	20.0	14	20.0	4	5.7
	Nearly disease-free...	89	92.7	62	64.6	2	2.1	51.6	41.3	20.0	68.3	51.7	31.1	26.5	85.2	15.3	22.4	10.2	8.4	14.8	7.2	52	61.9	20	23.8	10	11.9	2	2.4
	Average of moderately diseased		81.4 ±0.96		45.7 ±1.34		10.3 ±0.83	45.3	36.8	18.8	61.6 ±0.95	34.5 ±1.20	20.7	17.8	85.9	15.6	15.0	16.1	13.7	15.1	11.7	....	45.9 ±1.98	....	27.4 ±2.03	....	17.9 ±1.05	....	8.8 ±0.92
	Average of nearly disease-free		93.6 ±0.45		70.1 ±1.67		4.4 ±0.80	52.3	43.4	17.0	72.2 ±1.19	51.5 ±1.46	30.7	26.3	85.7	15.3	22.3	12.7	10.6	14.8	9.0	....	60.4 ±1.43	....	23.2 ±1.33	....	10.3 ±0.90	....	6.1 ±0.67
	Difference		12.2 ±1.06		24.4 ±2.14		5.9 ±1.15	....	....	....	10.6 ±1.52	17.0 ±1.89	....	....	....	....	....	....	....	....	....	....	14.5 ±2.44	....	4.2 ±2.43	....	7.6 ±1.38	....	2.7 ±1.14

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TABLE XXII.—*Comprehensive study of the principal data showing the influence of moderately diseased[a] and nearly disease-free[b] seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. Second planting (May 14) of the same series shown in Tables XXI, XXIII, and XXIV. Bloomington, Ill., 1921*

Soil plot No.	Condition of seed.	Early vigor data.						Yield data.																					
		Total stand.		Vigorous plants.		Weak plants.						Marketable corn.						Unmarketable corn.				Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling percentage.	Moisture percentage.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Moisture percentage.	Weight of dry matter.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Lbs.	*Lbs.*	*P. ct.*	*Bus.*	*Bus.*	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*		*Lbs.*								
1	Moderately diseased.	85	88.5	47	49.0	5	5.2	51.2	41.4	19.1	69.7	41.3	25.0	21.0	84.1	14.6	17.9	16.4	14.3	14.3	12.3	37	50.6	14	19.2	14	19.2	8	11.0
	Nearly disease-free...	87	90.6	54	56.3	3	3.1	52.0	42.4	18.5	71.0	46.8	27.8	24.0	86.4	15.4	20.3	14.6	12.3	14.5	10.5	42	56.0	24	32.0	6	8.0	3	4.0
4	Moderately diseased.	80	83.3	30	31.3	4	4.2	43.7	34.9	20.1	58.4	26.8	15.3	13.9	90.7	16.2	11.6	19.6	16.3	15.8	13.7	24	36.9	25	38.5	12	18.4	4	6.2
	Nearly disease-free...	94	97.9	68	70.8	2	2.1	57.1	48.4	15.2	77.8	49.2	30.8	25.8	83.8	17.6	21.3	17.6	14.8	16.4	12.4	43	53.7	22	27.5	10	12.5	5	6.3
7	Moderately diseased.	74	77.1	31	32.3	9	9.4	44.3	34.7	21.6	59.1	28.2	16.2	14.2	87.6	14.2	12.2	18.5	15.7	14.4	13.4	25	38.5	28	43.1	6	9.2	6	9.2
	Nearly disease-free...	90	93.8	71	74.0	4	4.2	46.8	37.8	19.2	63.5	40.4	24.0	20.8	86.7	15.9	17.5	13.8	11.8	15.3	10.0	34	53.1	20	31.2	9	14.1	1	1.6
9	Moderately diseased.	78	81.3	38	39.6	9	9.4	43.8	34.3	24.0	58.4	38.1	22.5	19.3	85.8	14.3	16.5	11.8	10.3	14.1	8.8	31	54.4	18	31.6	4	7.0	4	7.0
	Nearly disease-free...	92	95.8	68	70.8	1	1.0	54.0	44.1	18.3	74.1	53.3	31.8	27.2	85.6	15.0	23.1	12.3	10.5	14.4	9.0	45	60.8	17	23.0	8	10.8	4	5.4
10	Moderately diseased.	91	94.8	50	52.1	6	6.3	50.1	39.1	22.0	62.5	34.8	21.9	18.4	84.0	17.7	15.1	17.2	14.5	17.1	12.0	35	44.3	21	26.6	17	21.5	6	7.6
	Nearly disease-free...	91	94.8	69	71.9	7	7.3	55.2	44.6	19.2	71.8	47.8	30.0	25.1	83.7	17.4	20.7	14.6	12.3	15.3	10.4	46	57.5	16	20.0	7	8.8	11	13.7
13	Moderately diseased.	85	88.5	45	46.9	3	3.1	50.3	39.9	20.6	67.2	32.8	19.5	17.0	87.2	16.5	14.2	20.4	17.5	15.0	14.9	24	33.8	24	33.8	12	16.9	11	15.5
	Nearly disease-free...	90	93.8	68	70.8	5	5.2	56.6	46.5	17.8	73.6	58.8	36.7	30.7	83.7	17.0	25.5	9.8	7.6	15.8	6.4	56	66.6	15	17.9	6	7.2	7	8.3
16	Moderately diseased.	88	91.6	46	47.9	10	10.4	43.5	34.2	21.4	57.2	23.5	13.9	12.0	86.3	15.4	10.2	20.3	17.3	15.5	14.6	23	34.8	27	40.9	10	15.2	6	9.1
	Nearly disease-free...	92	95.8	77	80.2	6	6.3	51.6	41.7	19.2	68.0	41.5	25.5	21.6	84.8	16.5	18.0	16.2	13.8	16.6	11.5	44	51.7	19	22.4	15	17.7	7	8.2
19	Moderately diseased.	80	83.3	53	55.2	5	5.2	50.3	39.3	20.8	65.8	30.7	18.3	5.9	84.2	16.6	13.3	21.0	18.0	15.8	15.2	28	40.6	30	43.5	11	15.9	0	0
	Nearly disease-free...	87	90.6	72	75.0	4	4.2	51.2	40.4	21.1	67.6	41.1	25.1	21.3	84.9	16.3	17.8	15.3	13.2	15.3	11.5	43	54.4	19	24.0	10	12.7	7	8.9
22	Moderately diseased.	81	84.4	58	60.4	9	9.4	47.2	36.6	22.5	60.0	32.1	19.6	16.5	84.2	15.9	13.9	17.0	14.2	15.1	12.1	43	51.8	21	25.3	16	19.3	3	3.6
	Nearly disease-free...	91	94.8	78	81.3	3	3.1	54.7	43.4	19.6	71.1	53.1	32.5	27.2	83.1	15.6	23.0	10.9	9.0	13.8	7.8	54	66.6	13	16.1	9	11.1	5	6.2
	Average of moderately diseased		85.9 ± 1.16		46.1 ± 2.10		7.0 ± 0.59	47.2	37.2	21.2	61.9 ± 0.96	32.0 ± 1.18	19.1	16.5	86.0	15.7	13.9	18.0	15.3	15.2	13.0		42.8 ± 1.65		33.6 ± 1.83		15.9 ± 1.02		7.7 ± 0.93
	Average of nearly disease-free		94.2 ± 0.51		72.3 ± 0.96		4.1 ± 0.42	53.2	42.3	18.7	70.9 ± 0.89	48.0 ± 1.34	29.4	24.9	84.7	16.3	20.8	13.9	11.7	15.3	9.9		57.8 ± 2.20		23.8 ± 1.18		11.4 ± 0.70		7.0 ± 0.72
	Difference		8.3 ± 1.27		26.2 ± 2.38		2.9 ± 0.73				9.0 ± 1.31	16.0 ± 1.74											15.0 ± 2.35		9.8		4.5		0.7

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TABLE XXIII.—*Comprehensive study of the principal data showing the influence of moderately diseased[a] and nearly disease-free[b] seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. Third planting (May 21) of the same series shown in Tables XXI, XXII, and XXIV. Bloomington, Ill., 1921*

Soil plot No.	Condition of seed.	Early vigor data.						Yield data.																					
		Total stand.		Vigorous plants.		Weak plants.						Marketable corn.						Unmarketable corn.				Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling percentage.	Moisture percentage.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Moisture percentage.	Weight of dry matter.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Lbs.	*Lbs.*	*P. ct.*	*Bus.*	*Bus.*	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*		*Lbs.*								
1	Moderately diseased.	83	86.4	54	56.3	5	5.2	53.1	44.8	15.6	72.0	47.5	29.6	24.8	84.2	17.1	20.6	15.2	12.6	16.1	10.6	49	59.7	21	25.6	9	11.0	3	3.7
	Nearly disease-free...	94	97.9	85	88.5	1	1.0	55.3	46.2	16.5	75.2	51.0	30.8	26.3	85.4	16.0	22.1	15.4	12.4	15.4	10.5	45	53.6	26	30.9	12	14.3	1	1.2
4	Moderately diseased.	77	80.2	50	52.1	10	10.4	46.3	38.1	17.7	59.7	28.8	18.9	16.0	84.7	17.8	12.5	19.2	16.0	16.4	13.4	27	39.1	33	47.8	4	5.8	5	7.3
	Nearly disease-free...	81	84.4	71	74.0	6	6.3	51.7	43.9	15.1	70.6	52.8	32.5	27.5	84.5	16.8	22.9	11.4	9.1	15.8	7.7	49	65.3	19	25.4	4	5.3	3	4.0
7	Moderately diseased.	81	84.4	49	51.0	6	6.3	49.3	40.9	17.0	64.4	33.2	21.0	17.8	84.8	19.2	14.4	19.9	16.5	18.2	13.5	31	44.3	23	32.8	11	15.7	5	7.2
	Nearly disease-free...	90	93.8	69	71.9	2	2.1	47.7	38.6	19.0	64.2	40.4	23.7	20.4	86.5	14.3	17.5	14.9	12.0	14.6	10.3	38	51.3	26	35.1	7	9.5	3	4.1
9	Moderately diseased.	87	90.6	55	57.3	6	6.3	48.8	39.4	19.3	63.5	41.3	24.7	21.3	86.2	16.0	17.9	14.7	11.4	15.7	9.6	36	48.0	21	28.0	9	12.0	9	12.0
	Nearly disease-free...	88	91.6	67	69.8	2	2.1	56.2	47.7	15.1	74.8	52.4	33.7	27.8	82.5	18.4	22.7	14.0	11.7	17.1	9.7	55	63.9	18	20.9	10	11.7	3	3.5
10	Moderately diseased.	74	77.1	37	38.5	10	10.4	53.5	43.5	18.7	70.6	41.3	25.3	21.5	85.0	16.9	17.9	18.2	15.2	16.5	12.7	37	53.6	19	27.6	11	15.9	2	2.9
	Nearly disease-free...	86	89.6	60	62.5	3	3.1	57.6	48.1	16.5	79.9	55.2	33.0	28.3	86.0	15.7	23.9	15.1	12.8	16.4	10.7	50	63.3	16	20.2	12	15.2	1	1.3
13	Moderately diseased.	80	83.3	44	45.8	5	5.2	52.6	41.9	20.2	66.6	34.8	21.3	18.2	4.9	7.3	15.1	20.6	16.8	17.8	13.8	30	39.0	32	41.5	8	10.4	7	9.1
	Nearly disease-free...	87	90.6	69	71.9	2	2.1	58.1	48.1	17.2	77.3	53.1	33.1	28.2	85.2	18.4	23.0	15.0	12.7	17.3	10.5	49	59.7	22	26.8	2	2.5	9	11.0
16	Moderately diseased.	86	89.6	49	51.0	8	8.3	47.3	38.1	19.5	60.0	28.2	18.2	15.0	83.9	18.4	12.2	19.9	16.7	17.5	13.8	27	39.7	25	36.8	10	14.7	6	8.8
	Nearly disease-free...	91	94.8	70	72.9	1	1.0	52.3	43.2	17.4	67.2	42.5	27.2	22.6	83.2	18.6	18.4	16.0	13.1	18.1	10.7	45	54.9	20	24.4	16	19.5	1	1.2
19	Moderately diseased.	82	85.4	32	33.3	12	12.5	48.8	38.2	21.7	60.7	30.5	19.0	16.1	84.7	18.2	13.2	19.2	15.7	16.7	13.1	27	37.0	29	39.7	8	11.0	9	12.3
	Nearly disease-free...	91	94.8	65	67.7	3	3.1	50.7	41.1	18.9	67.6	46.4	27.9	23.6	84.6	16.5	20.1	13.2	11.0	16.5	9.2	46	63.0	14	19.2	13	17.8	0	0
21	Moderately diseased.	84	87.5	40	41.6	11	11.5	49.3	39.7	19.5	65.7	36.2	21.9	18.6	84.9	15.7	15.7	17.8	15.3	16.4	12.8	33	45.2	21	28.8	17	23.3	2	2.7
	Nearly disease-free...	82	85.4	57	59.4	8	8.3	51.2	41.8	18.4	68.0	43.8	26.8	22.9	85.4	17.0	19.0	15.0	12.5	16.4	10.5	53	64.6	15	18.3	12	14.6	2	2.5
	Average of moderately diseased..	....	84.9 ±0.92	....	47.4 ±1.75	....	8.4 ±1.89	49.9	40.5	18.8	64.8 ±0.94	35.8 ±1.38	22.2	18.8	84.8	17.4	15.5	18.3	15.1	16.8	12.6	....	45.1 ±1.61	....	34.3 ±1.61	....	13.3 ±1.04	....	7.3 ±0.77
	Average of nearly disease-free	....	91.4 ±0.95	....	71.0 ±1.74	....	3.2 ±1.65	53.4	44.3	17.1	71.6 ±1.13	48.6 ±1.15	29.9	25.3	84.8	16.9	21.1	14.4	11.9	16.4	10.0	....	60.0 ±1.13	....	24.6 ±1.20	....	12.2 ±1.19	....	3.2 ±0.65
	Difference.....	....	6.5 ±1.32	....	23.6 ±2.46	....	5.2 ±2.51	.....	.....	.....	6.8 ±1.47	12.8 ±1.79	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.9 ±1.22	....	9.7 ±2.01	....	1.1 ±1.58	....	4.1 ±1.01

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TABLE XXIV.—*Comprehensive study of the principal data showing the influence of moderately diseased [a] and nearly disease-free [b] seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. Fourth planting (May 30) of the same series shown in Tables XXI, XXII, and XXIII. Bloomington, Ill., 1921*

Soil plot No.	Condition of seed.	Early vigor data.						Yield data.																					
		Total stand.		Vigorous plants.		Weak plants.						Marketable corn.						Unmarketable corn.				Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling percentage.	Moisture percentage.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Moisture percentage.	Weight of dry matter.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Lbs.	*Lbs.*	*P. ct.*	*Bus.*	*Bus.*	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*		*Lbs.*								
1	Moderately diseased.	86	89.6	44	45.8	11	11.5	53.7	42.3	21.2	64.4	27.0	17.8	14.7	82.6	20.2	11.7	24.5	20.0	19.0	16.2	26	32.9	34	43.0	11	13.9	8	10.2
	Nearly disease-free...	91	94.8	65	67.7	1	1.0	58.4	47.0	19.5	80.3	61.8	35.5	30.2	85.0	16.3	26.8	11.5	9.5	15.6	8.0	55	67.9	14	17.3	7	8.6	5	6.2
4	Moderately diseased.	88	91.6	58	60.4	15	15.6	65.8	52.0	21.0	82.0	39.5	25.0	21.0	84.0	18.8	17.1	27.0	22.5	18.0	18.4	34	42.0	26	32.1	13	16.0	8	9.9
	Nearly disease-free...	90	93.8	78	81.3	2	2.1	70.3	59.6	15.2	92.8	63.7	41.9	34.6	82.6	20.3	27.6	17.7	14.8	15.1	12.6	56	62.9	25	28.1	4	4.5	4	4.5
7	Moderately diseased.	83	86.4	58	60.4	11	11.5	56.0	43.4	22.5	66.9	27.9	18.0	15.0	83.4	19.1	12.1	25.4	20.8	19.0	16.9	24	33.8	35	49.3	5	7.0	7	9.9
	Nearly disease-free...	95	99.0	72	75.0	3	3.1	60.6	49.9	17.7	76.7	46.2	30.1	25.0	83.1	19.9	20.0	19.8	16.4	19.4	13.2	46	52.9	24	27.6	13	14.9	4	4.6
9	Moderately diseased.	88	91.6	66	68.8	8	8.3	61.4	43.9	28.5	70.2	32.1	19.7	16.6	84.2	16.6	13.9	24.2	19.8	16.8	16.5	28	31.8	41	46.6	13	14.8	6	6.8
	Nearly disease-free...	87	90.6	71	74.0	4	4.2	68.7	56.1	18.3	85.1	62.3	41.8	34.3	82.0	21.2	27.0	14.3	12.0	17.3	9.9	54	63.5	18	21.2	10	11.8	3	3.5
10	Moderately diseased.	87	90.6	72	75.0	3	3.1	61.8	46.3	25.1	74.3	31.8	19.5	16.7	85.6	17.2	13.8	26.8	22.3	17.3	18.4	28	33.7	36	43.4	15	18.1	4	4.8
	Nearly disease-free...	89	92.7	76	79.2	5	5.2	67.0	53.9	19.5	85.3	64.8	40.6	34.2	84.2	17.8	28.1	13.3	10.8	17.6	8.9	56	66.7	12	14.3	13	15.5	3	3.5
13	Moderately diseased.	85	88.5	70	72.9	7	7.3	65.8	49.7	24.5	80.7	48.2	29.5	25.2	85.5	16.8	20.9	20.2	17.0	17.3	14.1	38	55.1	18	26.1	10	14.5	3	4.3
	Nearly disease-free...	85	88.5	77	80.2	3	3.1	64.2	51.1	20.4	81.7	50.3	31.2	26.5	84.9	17.7	21.8	19.9	16.6	18.3	13.6	45	50.0	29	32.2	9	10.0	7	7.8
16	Moderately diseased.	80	83.3	62	64.6	10	10.4	53.9	38.7	28.2	60.7	21.2	13.1	11.2	85.6	17.8	9.2	25.6	20.8	17.6	17.1	21	29.2	41	56.9	10	13.9	0	0.0
	Nearly disease-free...	85	88.5	76	79.2	2	2.1	63.2	51.4	18.7	79.4	54.5	34.9	29.2	83.7	19.0	23.6	16.5	13.4	19.6	10.8	50	61.0	15	18.3	13	15.8	4	4.9
19	Moderately diseased.	81	84.4	57	59.4	9	9.4	51.6	39.1	24.2	57.9	21.2	14.2	11.5	81.0	20.2	9.2	24.9	20.0	20.4	15.9	25	35.2	33	46.5	12	16.9	1	1.4
	Nearly disease-free...	82	85.4	63	65.6	6	6.3	52.2	41.2	21.1	64.4	41.3	26.2	21.8	83.2	18.0	17.9	15.0	12.2	18.1	10.0	41	58.6	20	28.6	8	11.4	1	1.4
21	Moderately diseased.	85	88.5	66	68.8	5	5.2	54.8	41.0	25.2	64.2	26.1	16.6	13.8	83.2	18.4	11.3	24.4	20.1	17.7	16.5	23	34.8	38	57.6	5	7.6	0	0.0
	Nearly disease-free...	85	88.5	70	72.9	2	2.1	56.2	44.0	21.7	69.0	49.6	31.5	26.2	83.2	17.9	21.5	12.5	10.2	17.6	8.4	51	66.2	12	15.6	10	13.0	4	5.2
	Average of moderately diseased		88.3 ±0.65		64.0 ±1.87		9.1 ±0.79	58.3	44.0	24.5	69.0 ±1.28	30.6 ±1.85	19.3	16.2	83.9	18.3	13.2	24.8	20.4	18.1	16.7		36.5 ±1.65		44.6 ±2.19		13.6 ±0.85		5.3 ±0.89
	Average of nearly disease-free		91.3 ±0.88		75.0 ±1.18		3.2 ±0.36	62.3	50.5	19.0	79.4 ±1.49	54.9 ±1.82	34.9	29.1	83.5	18.7	23.8	15.6	12.9	17.6	10.6		61.1 ±1.32		22.6 ±1.40		11.7 ±0.78		4.6 ±0.38
	Difference		3.0 ±1.09		11.0 ±2.21		5.9 ±0.87				10.4 ±1.96	24.3 ±2.59											24.6 ±2.11		22.0 ±2.60		1.9 ±1.15		0.7 ±0.97

| | | |

As the season advanced and conditions became more favorable for the growth of the weaker plants the difference in field stand became less, being only 3.0 per cent in the last planting (fig. 14).

TABLE XXV.—*Summary of data in Tables XXI to XXIV inclusive, showing percentage increases in favor of nearly disease-free seed over moderately diseased seed*

Date of planting.	Percentage increase in favor of nearly disease-free seed.				
	Total stand.	Vigorous plants.	Total yield.	Yield of marketable corn.	Marketable ears.
May 7	15.0	53.4	17.2	49.3	31.6
14	9.7	56.8	14.5	50.0	35.0
21	7.7	49.8	10.5	35.8	33.0
30	3.4	17.2	15.1	79.4	67.4

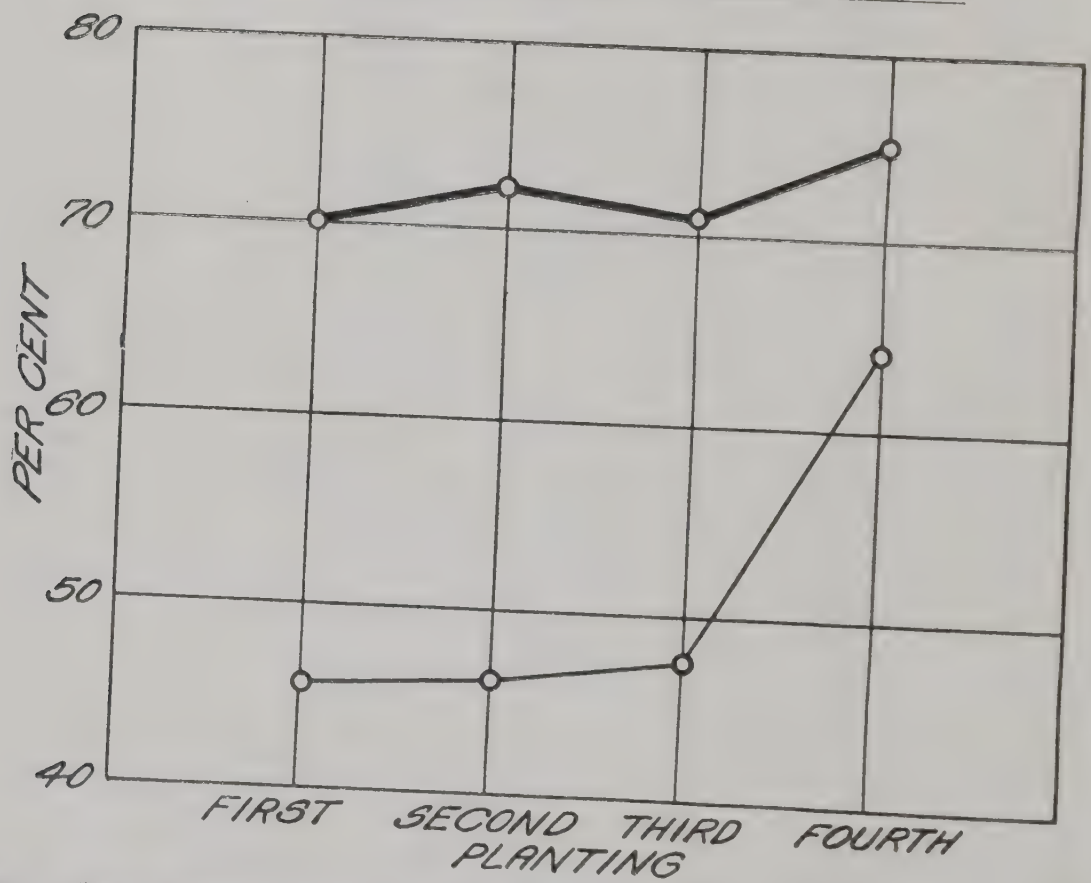

FIG. 15.—Graphic presentation of the percentage of kernels in the two lots of seed which produced vigorous plants when planted at different dates. The heavy line represents the nearly disease-free seed, the light light line the moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

From all these data it will be seen that reduction in percentage of field stand, due to the use of infected or susceptible seed, depends on several factors, among which are the extent of infection and the general condition of the soil in which the seed is planted. There seems to be little doubt that the planting of seed infected with or susceptible to the root and stalk rot pathogenes is responsible for many unsatisfactory

field stands and constitutes an important loss to the corn crop. This is especially true with corn planted early when weather and soil conditions are more or less unfavorable for germination and growth. However, a good field stand grown from diseased or disease-susceptible seed is no insurance against reduction in total yield. This fact is brought out in Table XXVI where the difference in field stand is only 3.0 per cent and the difference in total yield is 10.4 bushels per acre. It also occurs in Table XXIV where there is a difference of 3.4 per cent in field stand and 7.0 bushels in total yield. Moreover, in view of the results obtained by Kiesselbach and Ratcliff (25), it does not appear probable that the reductions in total field stand are an adequate explanation for all the reductions

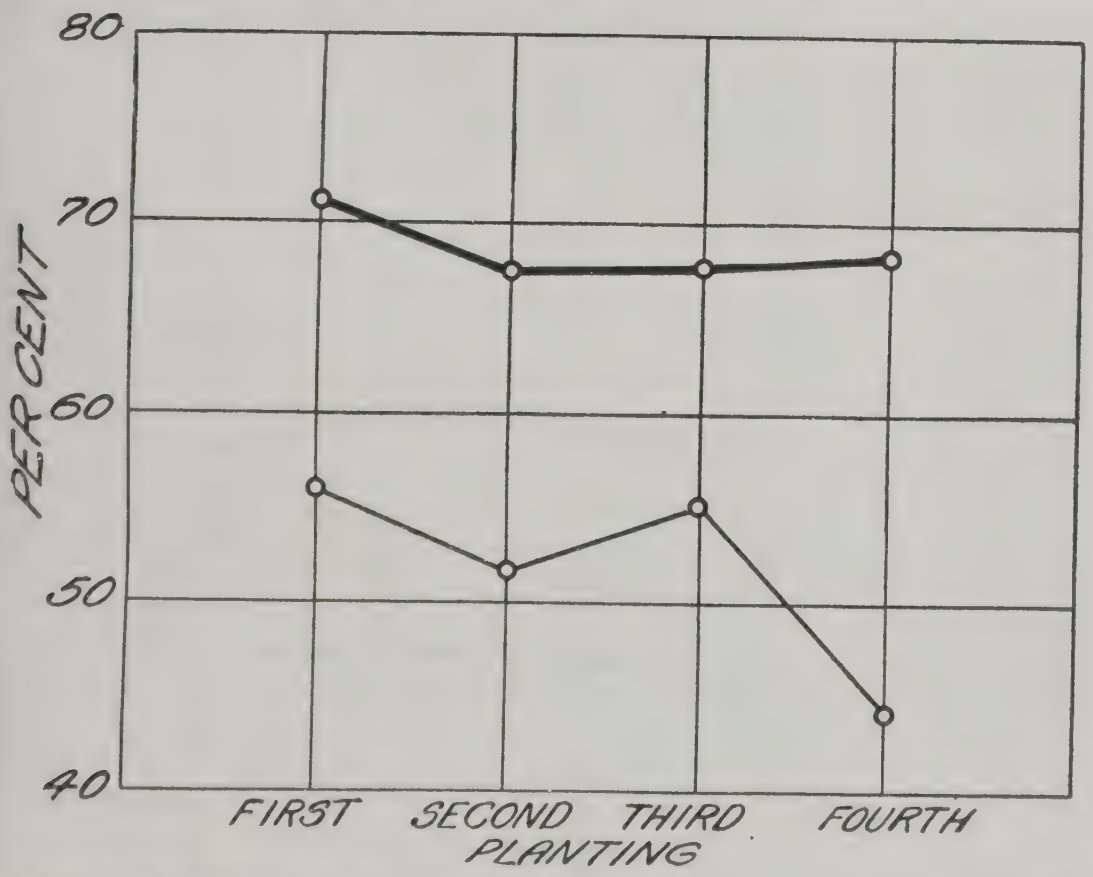

FIG. 16.—Graphic presentation of the percentage of marketable corn (by weight) produced by the two lots of seed at different dates of planting. The heavy line represents the nearly disease-free seed, the light line moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

in total yield in the experiments herein reported. An analysis of all the data shows that reduced stands may be considered, in a great majority of instances, an indication that a considerable portion of the corn population in question has grown from infected or susceptible seed which in turn is responsible for much of the reductions in total yield. The fact remains, however, that reductions in total yield herein reported can be traced to the use of infected seed and represent losses encountered in actual farm practice.

The reductions in early vigor in the corn grown from moderately diseased seed were well marked, with a very few exceptions, throughout all the experiments reported for 1921. The differences of 24.4 ± 2.14, 26.2 ± 2.38, 23.6 ± 2.46 and 11.0 ± 2.21 in percentage of vigorous plants

presented in Tables XXI to XXIV, and figures 9 to 12 and figure 15 are very significant. (Pl. 7.) Inasmuch as the majority of weak plants are either barren or nubbin producers, the differences in percentage of weak plants probably contributed to the wide differences in yield at the end of the season.

The differences of 17.0 ± 1.89, 16.0 ± 1.74, 12.8 ± 1.79, and 24.3 ± 2.59 bushels per acre of sound corn are greater than the differences in total yield and are of greater economic importance to corn growers. This is

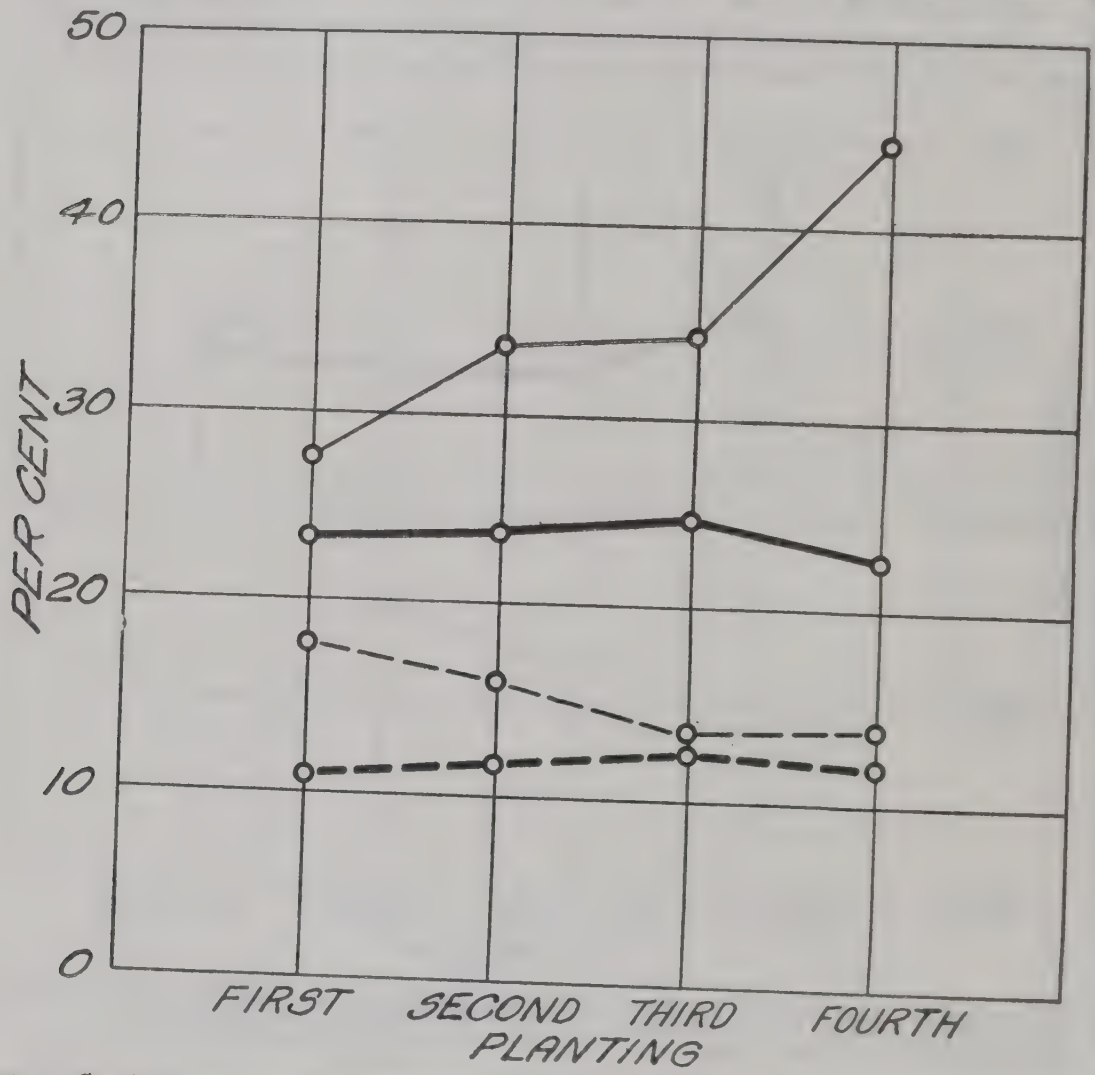

FIG. 17.—Graphic presentation of the total number of rotted ears (continuous lines) and the total number of chaffy ears (broken lines) produced by the two lots of seed at different dates of planting. The heavy lines represent the nearly disease-free seed, the light lines moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

especially true in seasons like 1921 when conditions were so favorable for the development of ear rots. The differences in the yield of marketable corn presented in Tables XXI, XXII, XXIII, and XXIV and figures 9, 10, and 11 are similar to those reported for 1920 and are well correlated with differences in early vigor (fig. 13). However, the results from the fourth planting that are presented in Table XXIV require some additional explanation. Although the difference in early vigor was the least in the last planting, yet the difference in yield of sound corn in this same planting was the greatest (Table XXV and fig. 12). The late-planted

corn suffered more from the combination of ear worm attack and weather conditions favorable to the development of ear rots. Both lots of corn were attacked approximately to the same extent by the earworms, but the corn grown from moderately diseased seed proved to be much more susceptible to the attacks of fungi following earworm injury than corn grown from nearly disease-free seed. Only 36.5 per cent of the ears from the moderately diseased seed were sound, while 61.1 per cent of the ears from the nearly disease-free seed were sound. Figure 16 presents graphically the percentage of marketable corn by weight produced by the moderately diseased and nearly disease-free seed from the different dates of planting.

A further analysis of the data, graphically presented in figure 17, shows that the increases in unmarketable corn in the later plantings from the moderately diseased seed were due to the increased number of rotted ears and not to any increase in number of chaffy ears. The decrease in the percentage of chaffy ears in the last three plantings from the moderately diseased seed is accounted for by the increased number of chaffy ears that were rotted. Ears and nubbins both rotted and chaffy were placed in the rotted class, since the rotting was considered more detrimental than the chaffiness. The decreased percentage of rotted ears in corn grown from the nearly disease-free seed as compared with that grown from the moderately diseased seed is very significant and indicates that the corn grown from nearly disease-free seed possessed considerably more resistance to injury by the fungi causing ear rots, as well as more resistance to the root and stalk rots, when planted in infested soil.

Additional data, similar to those in Tables XXI and XXII, are prepresented in Table XXVI. A comparison of the field performance of the corn planted from the three different seed lots, varying considerably in percentage of infection, shows that the diseased seed is associated with reduced field stand, reduced percentage of vigorous plants, increased percentage of weak plants, and reduced total yield, as well as reduced yield of sound, marketable corn. A considerable part of the increased yield of sound corn from the nearly disease-free seed is due to the smaller percentage of rotted ears.

TABLE XXVI.—*Experiment similar in purpose to that shown in Tables XXI to XIV, except that the corn was divided into three grades; moderately diseased (50.6 per cent diseased kernels), slightly diseased (10.6 per cent diseased kernels), and nearly disease-free (3.5 per cent diseased kernels). Planted May 10 on infested soil of medium fertility at some distance away from the series shown in the preceding tables. Bloomington, Ill., 1921.*

Soil plot No.	Condition of seed.	Early vigor data.						Yield data.																							
		Total stand.		Vigorous plants.		Weak plants.						Marketable corn.							Unmarketable corn.					Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling percentage.	Moisture percentage.	Weight of dry matter.	Test per bushel.	Weight before shelling.	Weight shelled.	Moisture percentage.	Weight of dry matter.	Test per bushel.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Lbs.	*Lbs.*	*P.ct*	*Bus.*	*Bus.*	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*		*Lbs.*	*Lbs.*								
1	Moderately diseased	110	91.7	96	80.0	5	4.2	60.3	54.0	10.4	73.7	51.5	37.7	32.2	85.4	13.4	27.9	55.5	16.3	13.8	13.2	12.0	50.0	59	62.8	24	25.5	1	1.1	10	10.6
	Slightly diseased	119	99.2	113	94.2	0	0.	70.1	61.7	12.0	85.8	57.6	41.2	36.0	87.4	13.3	31.2	57.5	20.5	17.6	12.9	15.3	51.5	65	60.7	26	24.3	9	8.4	7	6.6
	Nearly disease-free	118	98.3	109	90.8	1	.8	68.8	60.4	12.2	83.1	65.7	47.5	40.6	85.5	12.2	35.6	58.0	12.9	11.0	12.4	9.6	50.5	78	75.0	17	16.3	6	5.8	3	2.9
3	Moderately diseased	113	94.7	102	85.0	4	3.3	58.1	52.6	9.5	70.7	47.1	34.5	29.5	85.5	13.4	25.5	56.5	18.1	14.8	13.2	12.8	50.5	52	52.0	35	35.0	5	5.0	8	8.0
	Slightly diseased	117	97.5	102	85.0	1	.8	61.0	57.1	6.5	76.8	60.9	45.0	38.5	85.6	14.2	33.0	57.5	12.1	10.0	14.1	8.6	49.5	70	67.3	21	20.2	3	2.9	10	9.6
	Nearly disease-free	120	100.0	107	89.2	0	0.	67.2	61.2	8.9	83.1	65.9	48.2	41.3	85.7	13.6	35.7	58.5	13.0	10.7	12.9	9.3	53.0	77	73.3	18	17.1	5	4.8	5	4.8
5	Moderately diseased	112	93.3	90	75.0	2	1.7	58.6	52.6	10.2	73.1	46.1	33.1	28.4	85.8	11.8	25.0	55.0	19.5	16.4	11.0	14.6	51.5	50	55.6	25	27.8	3	3.3	12	13.3
	Slightly diseased	106	88.3	94	78.3	2	1.7	59.7	55.4	7.2	74.3	61.7	44.2	38.4	86.9	13.0	33.4	57.0	11.2	8.2	12.9	6.8	51.5	71	74.7	14	14.8	0	0.	10	10.5
	Nearly disease-free	115	98.8	102	85.0	3	2.5	68.0	62.5	8.1	82.1	68.9	49.5	42.9	86.7	13.0	37.3	58.5	13.0	9.6	12.4	8.4	49.5	79	74.6	17	16.0	3	2.8	7	6.6
6	Moderately diseased	101	84.2	79	65.8	9	7.5	60.7	56.3	7.3	76.2	50.0	37.3	31.4	84.2	13.6	27.1	55.5	19.0	16.4	13.6	14.2	51.0	60	61.9	24	24.7	5	5.2	8	8.2
	Slightly diseased	117	97.5	106	88.3	1	.8	57.8	51.4	6.4	75.1	51.7	37.4	31.6	84.5	11.8	28.0	57.5	16.7	14.4	11.8	12.7	51.0	65	66.3	28	28.6	1	1.0	4	4.1
	Nearly disease-free	112	93.3	108	90.0	2	1.7	71.9	66.6	7.4	92.0	65.0	47.4	40.2	84.8	12.5	35.2	58.0	19.2	16.6	12.3	14.6	53.5	77	68.7	26	23.2	3	2.7	6	5.4
7	Moderately diseased	102	85.0	77	64.7	4	3.3	56.5	49.9	11.7	69.8	44.1	31.9	27.0	84.9	11.4	23.9	56.5	18.0	15.7	11.6	13.9	53.0	51	54.3	22	23.4	4	4.3	17	18.0
	Slightly diseased	115	95.8	93	77.5	4	3.3	59.8	54.7	8.5	74.0	50.6	37.3	31.4	84.2	12.7	27.4	57.5	17.4	14.5	12.5	12.7	52.1	65	64.4	23	22.8	2	1.9	11	10.9
	Nearly disease-free	111	92.5	108	90.0	4	3.3	65.0	58.8	9.5	80.5	60.7	44.2	37.6	85.1	12.4	32.9	58.5	14.6	12.3	12.7	10.7	50.3	72	70.6	21	20.6	4	3.9	5	4.9
8	Moderately diseased	99	82.5	70	58.3	9	7.5	61.5	55.7	9.4	76.4	59.4	43.2	36.8	85.2	12.5	32.2	56.2	12.5	10.5	12.2	9.2	50.0	65	71.4	9	9.9	1	1.1	16	17.6
	Slightly diseased	105	87.5	67	55.8	8	6.7	68.0	61.7	9.3	84.5	61.8	45.1	38.3	84.9	12.5	33.5	58.0	16.6	14.0	12.0	12.3	52.5	74	66.7	23	20.7	1	.9	13	11.7
	Nearly disease-free	107	89.2	89	74.2	2	1.7	71.3	64.7	9.3	90.4	71.4	51.4	43.9	85.4	11.9	38.7	57.9	13.3	11.6	11.6	10.3	51.0	86	79.6	14	13.0	2	1.8	6	5.6
9	Moderately diseased	104	86.7	75	62.5	4	3.3	58.5	53.8	8.0	73.5	51.5	38.2	32.3	84.5	13.4	27.9	55.1	15.6	13.7	13.0	11.9	50.3	61	67.8	22	24.4	2	2.2	5	5.6
	Slightly diseased	107	89.2	86	71.7	4	3.3	62.3	56.2	9.8	77.3	60.0	44.0	36.9	83.8	12.0	32.5	57.9	12.2	10.7	11.9	9.4	55.8	79	80.6	7	7.2	2	2.0	10	10.2
	Nearly disease-free	112	93.3	98	81.7	4	3.3	69.0	62.9	8.8	85.8	63.1	46.8	39.3	83.9	13.1	34.2	58.5	16.1	14.1	12.9	12.3	53.1	74	69.2	24	22.4	2	1.9	7	6.5

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Some strains of Reid Yellow Dent have proved to be very susceptible to the root, stalk, and ear rot diseases when planted in infested soil or when the seed was inoculated at planting time with *Gibberella saubinetii*. Frequently ears of such strains of corn are so badly infected that it is impossible to select from them seed that is reasonably disease-free, regardless of the number of ears tested on the germinator. Seed from such a strain of Reid Yellow Dent was included in the inoculation experiments conducted in cooperation with Dr. James G. Dickson in 1921 on clean and infested soil, including early and late plantings. Corn grown from this seed proved to be very susceptible to injury by the organism (*Gibberella saubinetii*) with which it was inoculated. The corn in the inoculated plots had a slightly reduced stand and a much lower percentage of vigorous plants than in the uninoculated plots planted with the same lot of seed. The differences in percentages of strong plants in corn grown from this diseased, susceptible seed and in that grown from the nearly disease-free seed were very similar to those reported in Tables XXI to XXIV and XXVI. The very best seed that could be selected from this strain by use of the germination test averaged 27.5 per cent

TABLE XXVII.—*Total yield and yield of marketable corn as influenced by diseased,* [a] *susceptible,* [b] *and nearly disease-free* [c] *seed planted at different dates on clean soil and infested soil at Bloomington, Ill., in 1921*

Date of planting.	Condition of seed.	Total stand.		Yield data.									
					Marketable corn.			Unmarketable corn.					
		Number.	Per cent.	Total yield per acre.	Yield per acre.	Percentage by weight.	Test per bushel.	Yield per acre.	Percentage by weight.	Test per bushel.	Marketable ears.	Rotted ears and rotted nubbins.	Chaffy ears and chaffy nubbins.	Sound nubbins.
				Bus.	*Bus.*		*Lbs.*	*Bus.*		*Lbs.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
INFESTED SOIL														
May 10	Original composite of susceptible seed [a]	312	86.7	77.6	48.0	61.8	54.5	29.6	38.2	48.7	53.9	34.6	2.2	9.3
	Selected composite of susceptible seed [b]	333	92.5	78.3	50.0	63.9	54.2	28.3	36.1	47.7	54.2	34.9	2.5	8.5
	Nearly disease-free composite [c]	354	98.3	89.7	69.3	77.2	58.5	20.4	22.8	51.5	71.3	17.7	3.2	7.8
30	Original composite of susceptible seed	320	88.8	76.5	37.7	49.3	52.0	38.8	50.7	49.0	45.5	42.0	4.2	8.3
	Selected composite of susceptible seed	334	92.8	71.8	39.7	55.3	52.5	32.1	44.7	49.2	49.5	40.5	2.4	7.6
	Nearly disease-free composite	354	98.3	81.5	59.3	72.7	56.5	22.2	27.3	51.3	68.5	25.0	1.3	5.2
CLEAN SOIL														
11	Original composite of susceptible seed	319	88.6	81.0	51.8	63.9	53.5	29.2	36.1	49.2	54.7	30.8	6.9	7.6
	Selected composite of susceptible seed	336	93.4	75.7	42.5	56.1	54.5	33.2	43.9	48.7	48.0	32.5	8.8	10.7
	Nearly disease-free composite	346	96.1	90.6	73.0	80.5	58.3	17.6	19.5	50.8	71.1	19.0	1.9	8.0
28	Original composite of susceptible seed	332	92.1	94.9	53.9	56.8	53.6	41.0	43.2	49.7	50.2	38.0	3.5	8.3
	Selected composite of susceptible seed	345	95.8	95.4	55.2	57.8	53.8	40.2	42.2	50.0	51.5	37.0	4.0	7.5
	Nearly disease-free composite	353	98.2	104.7	81.6	77.9	57.8	23.1	22.1	52.6	73.9	16.2	1.0	8.3

[a] 96.5 per cent viable and 38.6 per cent diseased.
[b] 98.9 per cent viable and 27.5 per cent diseased.
[c] 99.9 per cent viable and 3.5 per cent diseased.

diseased. Although the complete data and discussion regarding these experiments are reserved for other publications, a limited number of these data are presented in Table XXVII to show the influence of seed infection and susceptibility to the root and stalk rots to total yield and yield of marketable corn, with special reference to the percentage of rotted ears.

The field stand data (Table XXVII) are of particular interest. Many investigators would consider a field stand of 92.5 to 95.8 per cent a satisfactory stand. These stand data lend further emphasis to the fact that satisfactory field stands are no guarantee against losses due to the root, stalk, and ear rot diseases.

TABLE XXVIII.—*Summary of data presented in Table XXVII, showing percentages of rotted ears and yield of marketable corn*

Condition of seed.	Early planting.		Late planting.	
	Yield of marketable corn.	Rotted ears and nubbins.	Yield of marketable corn.	Rotted ears and nubbins.
	Bushels.	*Per cent.*	*Bushels.*	*Per cent.*
INFESTED SOIL				
Selected composite of susceptible seed	50.0	34.9	39.7	40.5
Nearly disease-free composite	69.3	17.7	59.3	25.0
CLEAN SOIL				
Selected composite of susceptible seed	42.5	32.5	55.2	37.0
Nearly disease-free composite	73.0	19.0	81.6	16.0

Although there was a consistent reduction in total yield in the corn grown from the diseased, susceptible seed, the greatest and most important differences were in the yields of sound, marketable corn and the percentages of rotted ears. These items are summarized in Table XXVIII and figures 18 and 19. In both plantings and on both infected and clean soil the corn grown from diseased, susceptible seed produced more rotted ears and a much lower yield of marketable corn. The bushel weights indicate that the marketable corn produced by the nearly disease-free seed was of better quality than that produced by the diseased, susceptible seed.

The results secured at Urbana, reported in Table XXIX and figure 20, were obtained from corn grown from the same moderately diseased and nearly disease-free seed composites as the date-of-planting experiments conducted at Bloomington, reported in Tables XXI to XXV. The soil, however, was much higher in fertility than that of the Bloomington plots; in fact, the Urbana soil was in a better state of balanced fertility than the average Corn Belt soil will be for several years. The standards for classifying the plants into vigorous, semivigorous, and weak were based entirely on height measurements. Plants from the moderately diseased seed were grouped into three approximately equal divisions according to height. This same standard when applied to the plant populations from nearly disease-free seed gave relatively high percentages of vigorous plants and low percentages of weak plants.

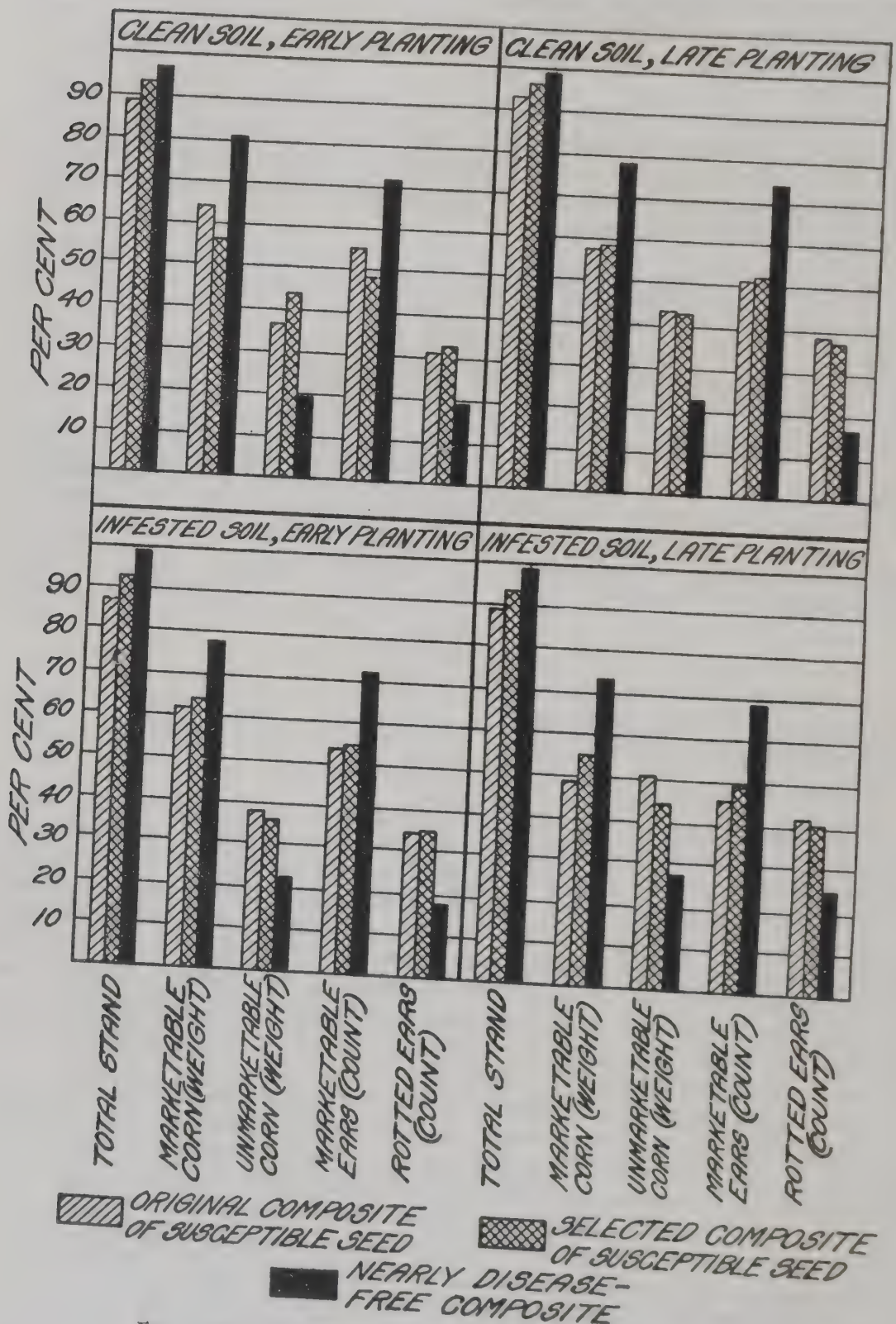

FIG. 18.—Graphic presentation of data presented in Table XXVIII.

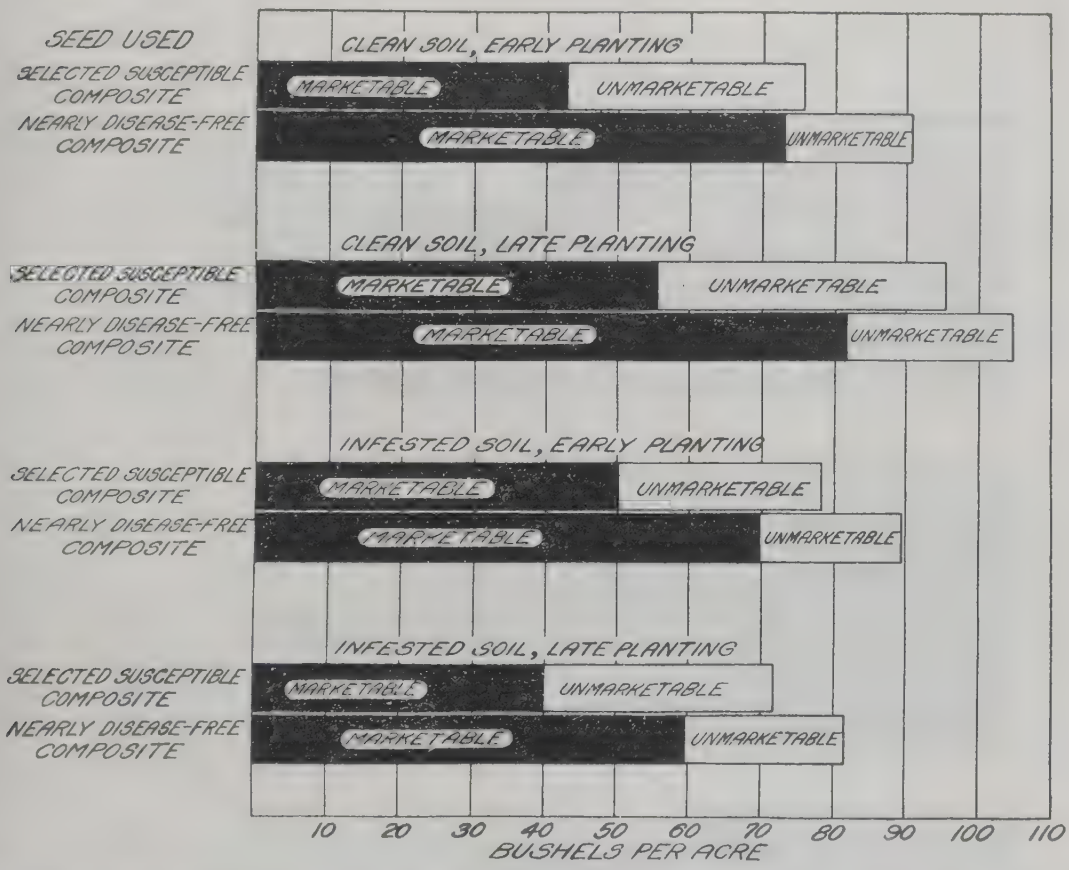

FIG. 19.—Graphic illustration of the total yield and yield of marketable and unmarketable corn (in bushels per acre) produced by a selected composite of susceptible seed and by nearly disease-free seed. (Taken from Table XXVII.)

TABLE XXIX.—*Influence of an early date, two intermediate dates, and a late date of planting of moderately diseased and nearly disease-free seed on early vigor and yield. Reid Yellow Dent grown on infested soil of high fertility. Urbana, Ill., in 1921*

Time of planting.	Condition of seed.	Early-vigor data.						Yield data.									
		Total stand.		Vigorous plants.		Weak plants.		Total yield per acre.	Marketable corn yield per acre.	Marketable ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.			Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
								Bus.	*Bus.*								
May 2	Moderately diseased	1,464	86.8	352	21.6	681	39.1	90.9	73.4	902	67.2	174	13.0	130	9.7	136	10.1
	Nearly disease-free	1,599	95.2	914	58.1	123	6.4	100.7	89.6	1,110	76.1	114	7.8	80	5.5	154	10.6
10	Moderately diseased	1,448	85.7	385	24.9	513	28.2	86.3	69.4	790	64.3	170	13.9	128	10.4	140	11.4
	Nearly disease-free	1,626	96.4	1,362	81.1	88	5.0	99.8	85.2	1,022	72.3	138	9.8	128	90.5	126	8.9
20	Moderately diseased	1,477	88.7	578	34.2	410	21.5	77.0	57.4	746	58.1	190	14.8	170	13.2	178	13.9
	Nearly ;disease-free	1,593	94.8	1,028	61.5	211	13.5	90.5	79.1	1,004	73.2	128	9.3	96	7.0	144	10.5
31	Moderately diseased	1,691	88.2	496	25.8	452	23.9	79.0	55.9	860	61.4	192	13.7	222	15.9	126	9.0
	Nearly disease-free	1,784	92.9	1,199	65.5	55	2.9	89.9	76.6	1,144	72.9	166	10.6	114	7.3	144	9.2

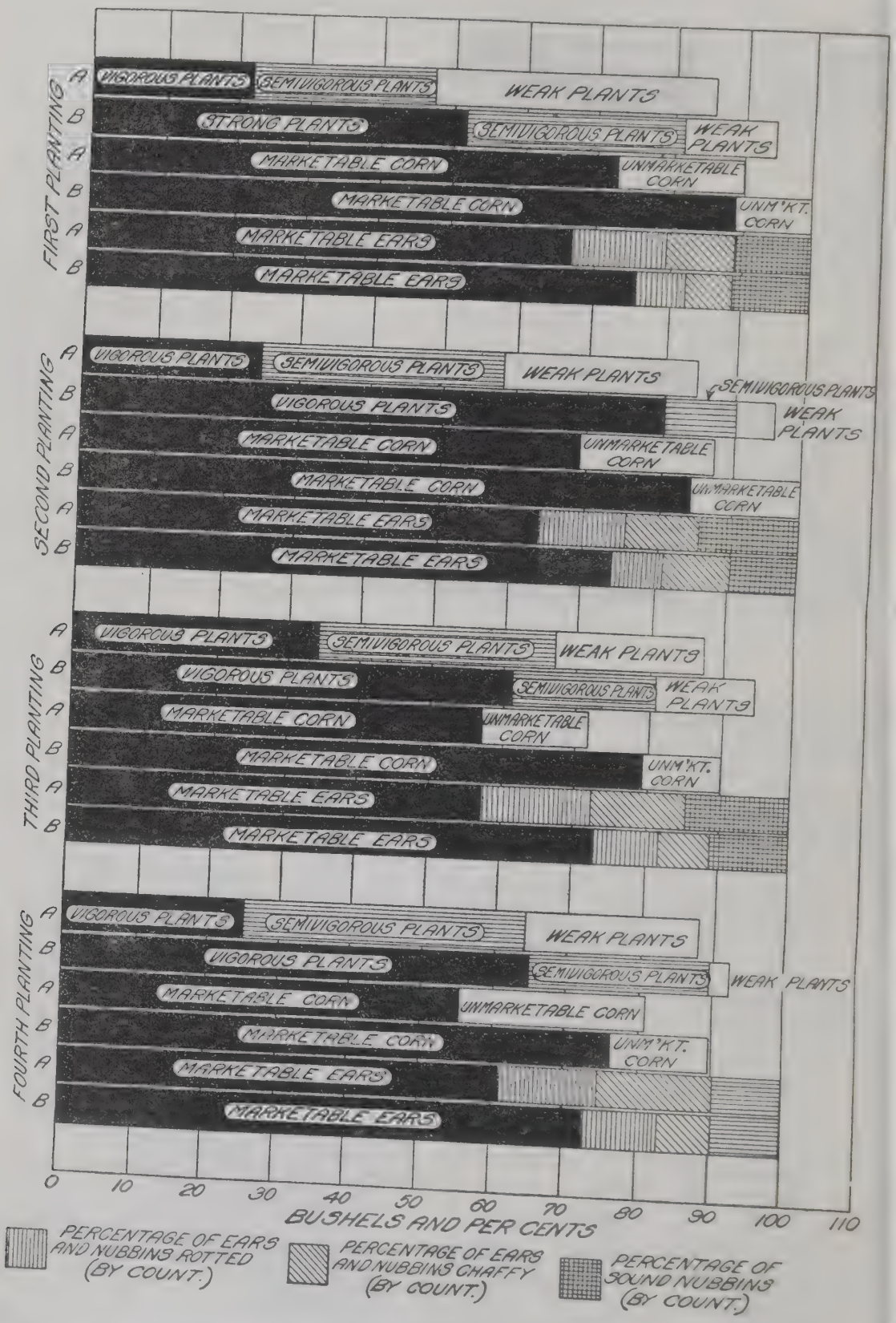

FIG. 20.—Graphic summary of Table XXIX, showing comparative results from planting moderately diseased (A) and nearly disease-free (B) seed on four different dates. Upper pair, vigor of seedlings (in percentages); middle pair, relation of seedling vigor to acre yield in bushels of marketable and unmarketable shelled corn; lower pair, comparative percentages by number of marketable ears, rotted ears and nubbins, chaffy ears and nubbins, and sound nubbins.

SUMMARY

1. Studies were conducted with diseased and apparently disease-free seedlings, transplanted from the germinator to the field. The disease-free seedlings made more rapid increases in height and circumference than the diseased seedlings during the first 25 days. The plants developed from the disease-free seedlings gave a much higher yield of grain than the plants from diseased seedlings.

2. Approximately 153,000 corn plants were classified during their early growth stages as vigorous, semivigorous, or weak. Of this number, more than 6,000 were harvested individually and the plant yields determined. The populations were grown from both diseased and relatively disease-free seed.

3. Early height and yield of corn plants were found to be correlated directly. In most cases the coefficient of correlation was sufficiently large to be significant.

4. Plants strong and vigorous in their early stages of growth produced a larger percentage of good-sized ears, and therefore higher yields of grain, and matured their grain somewhat earlier than the weaker ones, regardless of height at harvest time.

5. Plants weak in their early stages of growth usually produced nubbins only, or were barren.

6. Yields were recorded on the basis of marketable and unmarketable corn, the latter consisting of small nubbins, rotted ears, and light chaffy ears.

7. Nearly disease-free and moderately diseased seed were compared under different conditions in a number of experiments at Bloomington, Urbana, and elsewhere in Illinois.

8. Corn grown from seed not infected with the root and stalk rot pathogenes or not susceptible to the root and stalk rots had a higher percentage of strong, vigorous plants, a lower percentage of weak plants, and produced higher total yields and higher yields of marketable corn than corn grown under comparable conditions from infected or susceptible seed.

9. Corn populations grown from seed infected with one or more of the root and stalk rot pathogenes or susceptible to the root and stalk rots produced a much lower yield of sound, marketable corn and a larger percentage of nubbins, rotted ears, and light chaffy ears than corn grown from seed relatively free from and resistant to infections.

10. Corn earworms seem to show little preference between corn from diseased or nearly disease-free seed within a variety, but the total injury on the latter is much less because the worm injury is not followed by ear rots to the extent it is on the former.

11. Relatively disease-free seed ears selected from badly rotted plants proved to be inferior to relatively disease-free seed ears selected from apparently disease-free plants, especially when the comparisons were made on infested soil.

12. Not all lots of seed corn are suitable for selecting disease-free seed corn. Some types of corn are so susceptible to disease that seed which passes a good test on the germinator proves very susceptible when planted in infested soil.

13. Corn resistant to root and stalk rots is also resistant to ear rots.

CONCLUSIONS

1. Early vigor and yield of the corn plant are closely correlated.
2. Variations in early vigor are due, not simply to chance, but to certai factors, genetic, physiologic, and pathologic, which are more or less unde the control of the investigator, the corn breeder, and the corn grower.
3. The importance of the disease factor demands that it be given prominent place in any program which aims at the permanent improve ment of the corn crop.

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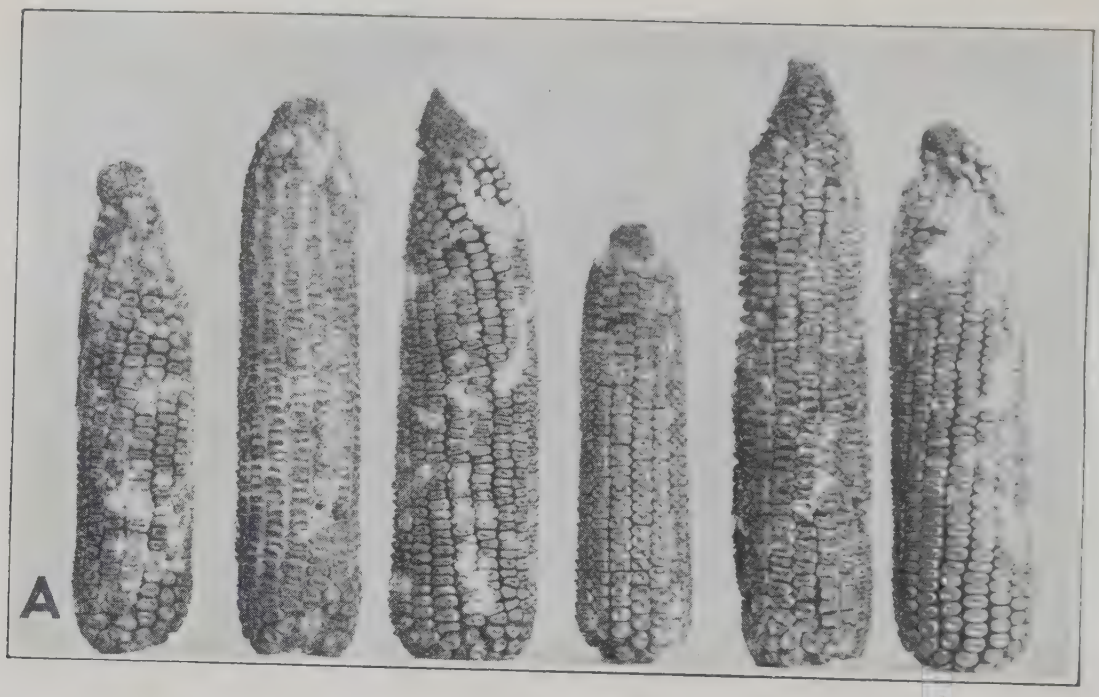
PLATE 1

Characteristic appearance of vigorous and weak young corn plants.
A.—A healthy, vigorous plant, grown from disease-free seed.
B.—A weak plant, stunted in size, and somewhat chlorotic, grown from diseased seed.
C.—A weak plant, chlorotic and partly blighted, grown from diseased seed.
D.—A weak plant, very chlorotic and nearly dead, grown from diseased seed.
All plants were of the same age and were drawn to the same scale.

D
C
B
A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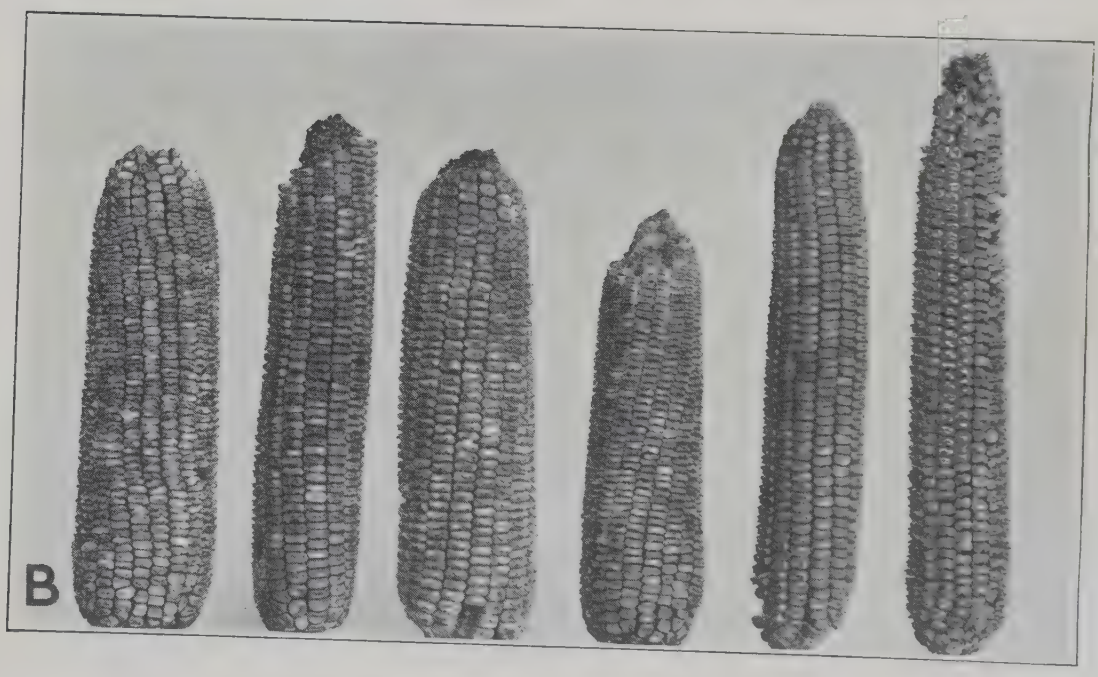
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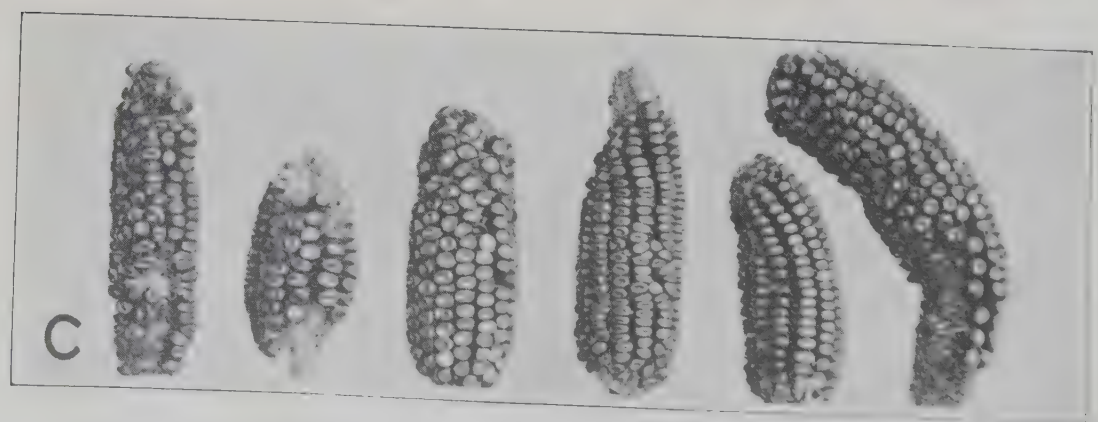

C

PLATE 2

Representative ears of unmarketable corn.

A.—Rotted ears. This classification includes not only totally rotted ears but also those that are partly rotted. Ears of the latter kind may be composed largely of good kernels, but when shelled the rotted portion would greatly reduce the market grade of the whole.

B.—Chaffy ears. There is some space between the kernels so that the ears can easily be bent and twisted. Such kernels are light and poorly developed and consequently have little market value. Chaffy ears that also showed signs of rot were placed in the rotted class shown in A because the rotting is more detrimental.

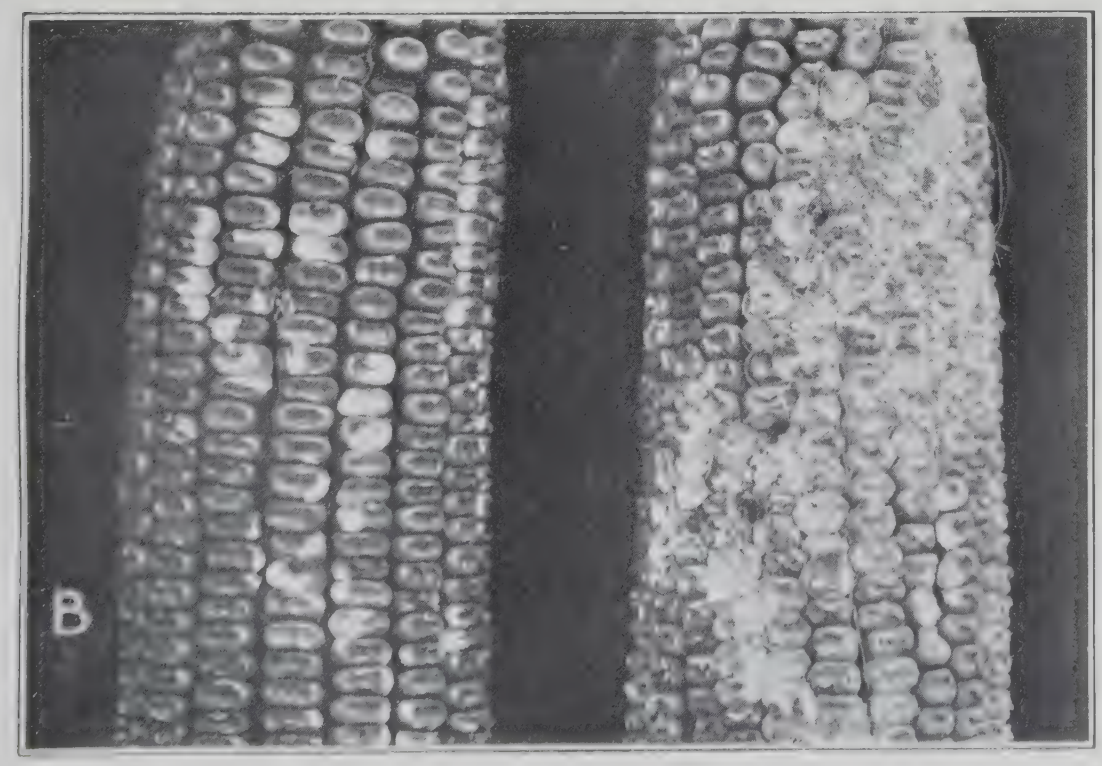
C.—Nubbins. Although most of the kernels here shown would be good for feeding purposes, most of these nubbins would be missed by the average corn husker. Furthermore, a large percentage of nubbins is produced by weak diseased plants.

26551—23——4

PLATE 3

A.—Representative ears of marketable corn. Compare with unmarketable corn, Plate 2.

B.—On the left, an ear of a resistant type grown from disease-free seed and resistant to ear rots. On the right, an ear grown from diseased seed and susceptible to ear rots. Both ears were injured by the ear worm approximately to the same extent. On the resistant type (left) the injury was confined to that done by the worm, which injury was practically negligible as far as marketing is concerned. On the other ear (right), susceptible to ear rots, the worm injury was followed by fungous rots which made it practically worthless.



Washington, D. C

A
B

PLATE 4

Total yield from moderately diseased and disease-free seed of Reid Yellow Dent planted simultaneously in two parallel, 10-hill plots, at Bloomington, Ill.

A.—Product from 10 hills grown from moderately diseased seed. Ears seventh and eighth (from left) were chaffy, ninth and twelfth were partly rotted, and fifteenth was a nubbin.

B.—Product from 10 hills grown from disease-free seed. All these ears would be considered marketable.

PLATE 5

A.—Two hills of corn of same age, photographed 44 days after planting. The hill on the left contains two plants from diseased seed, the hill on the right three plants from disease-free seed.

B.—Same hills as in A, photographed 120 days after planting. The hill on the left produced 380 gm. and the hill on the right 930 gm. of air-dry shelled corn.

A
B

A

B

PLATE 6

Adjacent plots of Reid Yellow Dent planted at the same time, on uniform soil, and photographed on the same day, at Urbana, Ill.

A.—Plot grown from moderately diseased seed. This plot contained a large percentage of weak plants.

B.—Plot grown from nearly disease-free seed. This plot had 55 per cent more vigorous plants than the plot shown in A.

PLATE 7

Adjacent plots of Reid Yellow Dent planted at the same time, on uniform soil, with rows the same distance apart and same number of kernels per hill, and photographed on the same day, at Bloomington, Ill.

A.—View down the center of four rows planted with moderately diseased corn.

B.—View down the center of adjacent four rows planted with nearly disease-free corn.

Note the increase in size and vigor of the plants in B. Note that in A leaves barely meet between the rows in the center of the pircture while in B they overlap considerably.

A

B

EFFECT OF BURNING ON VEGETATION IN KANSAS PASTURES [1]

By R. L. HENSEL [2]

Pasture Specialist, Kansas Agricultural Experiment Station

Burning pastures in the spring is a common practice on many Kansas farms. Those who practice burning contend that it reduces weeds, provides green feed at an earlier date and insures a better distribution of stock and more uniform grazing. It is also contended that no injury to the grass results. On the other hand, there are those who oppose burning on the ground that it does not cause growth to start earlier and that it injuries the pastures by encouraging the growth of weeds and by decreasing the stand of desirable grasses. They admit that burned areas appear green earlier but maintain that the difference is apparent rather than real, for if a close examination is made it will be found that there are as many green plants on the unburned areas as on the burned. The accumulated surface litter covers the green plants in the former case.

No doubt the practice of grass burning is a relic of Indian days when it was customary for the different tribes to burn off certain, well-chosen areas in the spring. The main object apparently was to obtain fresh, green feed early in the year. Moreover, the areas made greener by burning would more likely attract game animals and hunting would be made easier.

This practice was perhaps passed on to the earlier settlers and has been continued to the present day. The objects now, however, are different. Today, one of the principal objects of burning pastures is to provide inexpensive forage for live stock early in the season, thereby eliminating the cost of the more expensive feed. In addition to this, there are always areas in the larger pastures which for various reasons are not fully grazed. The grass in these places grows rank and becomes unpalatable. Burning destroys this dead, unpalatable grass and permits the new growth to be grazed to better advantage. No experimental evidence relating to the effect of burning appears to be available, although it is clear that such information is needed in view of the importance of the question and the controversial opinions regarding it.

In order to secure such information, the Kansas Agricultural Experiment Station began a series of studies in 1918, which were designed to secure data on the question. These experiments were conducted about 9 miles north of Manhattan on a 1,500-acre tract of pasture land.[3] The

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[1] Accepted for publication Aug. 18, 1922. Contribution No. 142 from the Department of Agronomy, Kansas Agricultural Experiment Station.

[2] The experiments discussed in this paper were inaugurated in 1918 by Mr. R. K. Bonnett. In 1919, Mr. C. W. Mullen had charge of the work until July 15. Since that time the work has been in charge of the author.

[3] Mr. Dan D. Casement of Manhattan cooperates with the Agricultural Experiment Station in the study of certain pasture problems on this tract of land.

land is typical eastern Kansas prairie. Using Clements'[4] classification, the vegetation belongs in the "True Prairie" (Stipa-Koeleria) association.

TOPOGRAPHY AND SOIL

The topography is rolling and the land is too hilly for cultivation. The soil ranges from a dark brown to black loam with an underlying subsoil composed of clays and shales. There are many outcroppings of limestone, and many of the slopes and ridges are covered with loose chert rock. The elevation varies from 1,200 to 1,400 feet above sea level.

CLIMATIC CONDITIONS

The average, annual precipitation based on a 59-year period preceding 1918 is 31.50 inches. Table I shows the monthly precipitation at Manhattan for the duration of these experiments—namely, from 1918 to 1921 inclusive. It will be noticed that the rainfall during each of these years was less than the 59-year average, indicating that conditions, so far as rainfall was concerned, were subnormal but probably insufficient to influence the experiments to any marked degree.

The average date of the last killing frost in the spring at Manhattan is April 25 and that of the first killing frost in the fall is October 8, both based on the 59-year period. The average frost-free period is, therefore, 166 days. The growing season for the principal native grasses is much longer, however.

TABLE I.—*Precipitation by months at Manhattan, Kans., from 1918 to 1921 and the 59-year average*

Month.	Precipitation.				
	1918.	1919.	1920.	1921.	1859 to 1917 (average).
	Inches.	*Inches.*	*Inches.*	*Inches.*	*Inches.*
Jan.	0.65	0.26	0.13	1.08	0.81
Feb.	.52	1.24	.55	.36.	1.26
Mar.	.76	5.03	.20	.16	1.46
Apr.	4.60	3.40	4.09	2.95	2.56
May.	5.28	3.15	1.75	3.32	4.63
June.	1.56	4.66	2.24	6.26	4.57
July.	1.98	1.45	4.83	4.21	4.67
Aug.	3.34	1.43	6.76	4.65	3.66
Sept.	2.35	2.65	4.39	3.17	3.34
Oct.	4.87	.68	.90	1.38	2.26
Nov.	1.55	2.62	2.20	0.00	1.37
Dec.	2.30	.08	1.35	0.00	.91
Total.	29.76	27.65	28.39	27.54	31.50

The principal growth takes place from March 15 to July 1. If rainfall is deficient in March, April, and May a "backward" season results, but this may be made up by heavy rains in July and August, which will start vigorous growth, resulting in abundant late summer pasture.

[4] CLEMENTS, Frederic E. PLANT INDICATORS. xvi, 388 p., 25 fig., 92 pl. Washington, D. C. 1920. Bibliography, p. 364-373. (Carnegie Inst. Wash. Pub. 290).

VEGETATIVE COVER

The principal grasses are big bluestem (*Andropogon furcatus* Muhl.), little bluestem (*Andropogon scoparius* Michx.), side oats grams (*Bouteloua curtipendula* (Michx.) Torr.), and Kentucky bluegrass (*Poa pratensis* L.). Grasses of lesser importance are blue grama (*Bouteloua gracilis* H. B. K.), hairy grama (*Bouteloua hirsuta* Lag.), sand drop seed (*Sprorobolus cryptandrus* (Torr.) Gray), switch grass (*Panicum virgatum* L.), and Indian grass (*Sorghastrum nutans* (L.) Nash). Common weeds are annual ragweed (*Ambrosia artemisifolia* L.), perennial ragweed (*Ammbrosia psilostachya* D. C.), prairie sage (*Artemesia gnaphaloides* Nutt.), prairie ragwort (*Senecio plattensis* Nutt.), dwarf fleabane (*Erigeron divaricatus* Michx.), snow-on-the-mountain (*Euphorbia marginata* Pursh.), and others of lesser importance. There is considerable wolfberry (*Symphoricarpos occidentalis* Hook.) on some of the overgrazed pastures. Two sedges, *Carex pennsylvanica* Lamarck and *Carex meadii* Dewey, are common over the entire region.

EXPERIMENTAL METHODS

The experiments were planned to obtain data on the following points:

1. The effect of burning on the temperature of the soil during the early growing season (March 15 to June 10) at depths of 1 and 3 inches below the surface.
2. The effect of burning on grasses.
3. The effect of burning on weeds.
4. The effect of burning on the yield.

The experiments were conducted over an area 75 by 150 feet on a ridge top with a slight southern slope. The area was divided into two sections. One of these, called section A, was burned as early each year as conditions would permit and the other, section B, was never burned.

Section A (Pl. 1, A) was burned March 23, 1918, March 13, 1919, April 2, 1920, and April 7, 1921, the variations in dates being due to seasonal conditions. The burning was usually conducted shortly after a rain in order to avoid possible injury to the roots and also to afford better control of the fire.

The number of plants of each species of grasses, sedges, and weeds, the temperature of the soil, the time at which growth began in the spring each year, and the yield of forage was determined for each area. The yields were generally determined in September of each year. The accumulated growth was cut and removed from the plots (Pl. 1, B) immediately after. It was planned to leave as much material as is usually left by livestock in order to allow the plots to remain in a condition comparable to a grazed area, although it might have been better to have turned in a few head of livestock. However, this would have disturbed the permanently staked quadrats and probably would have interfered with the main purpose of the experiments.

It was originally planned to take soil samples on each plot at regular intervals, but this could not be done because of the rocky soil of the area.

DETERMINING THE TEMPERATURE

The four instruments used for obtaining soil temperatures were Columbia recording thermographs fitted with soil bulbs. Two were on the plot which was burned each year; one recorded temperatures at 1

inch under the surface and the other at 3 inches. Two others on the plot which was never burned also recorded the temperature at depths of 1 and 3 inches. The thermographs were kept in operation continuously from the time the field work began early in the spring until the latter part of June or the first of July.

QUADRAT CHARTINGS

In order to study the effect which the different treatments had on the principal plants, 7 small quadrats, each 10 inches square, were laid out in each plot. (Pl. 2, A.) The quadrats were bounded by a wooden frame, across which, in both directions, were woven strands of ordinary fine twine, held in place by small nails driven into the frame. These were placed at intervals of one-half inch. The twine divided each quadrat into 400 squares, each one-half inch on a side. These frames were permanently staked down so that exactly the same area would be charted each time. Sheets of paper similarly divided were used for recording the data, and upon these the observer placed the exact composition as he found it in each square of the quadrat. On account of the small size of the squares, it was not necessary to sketch the size of each grass tuft (Pl. 2, B). Instead, the results were compiled by squares for grasses and sedges and by specimens for the weeds. For example, quadrat A, 1 might contain 150 squares of big bluestem, 75 of little bluestem, and 15 specimens of dwarf fleabane. The method of using the squares as the basis of measurement, rather than the exact area occupied by each plant is not generally recommended, but in this case it was used, not only because it simplified the work but also because of the small size of both the individual grass plants and the squares on the quadrat frame. It has proved successful and has shown the relative change from year to year in a satisfactory way.

YIELDS

In order to arrive at the difference in amount of forage actually produced on the different plots, a number of cuttings were made on each area. This was generally done in September. An iron hoop, the rim of which included an area of one ten-thousandth of an acre, was used. The hoop was permitted to fall more or less at random and the material within the borders cut 1 inch above ground with a pair of sheep shears. The material was then dried and weighed and the yields per acre calculated.

EXPERIMENTAL RESULTS

THE EFFECT OF BURNING ON GRASSES

Quadrats on both the early-burned area and the unburned area were charted three times each season. From this data the number of squares which contained grasses, sedges, and weeds was compiled. A summary of the data on grasses and sedges is given in Table II. The dates of burning were March 23, 1918, March 13, 1919, April 2, 1920, and April 7, 1921.

TABLE II.—*Number of squares on all quadrats containing grasses and sedges (1918 to 1921)*

Date of charting.	Burned area.			Unburned area.		
	Grasses.	Sedges.	Both.	Grasses.	Sedges.	Both.
First:	*Squares.*	*Squares.*	*Squares.*	*Squares.*	*Squares.*	*Squares.*
Apr. 9, 1918	234	87	321	21	34	55
Apr. 17, 1919	308	114	422	163	75	238
May 5, 1920	621	39	660	492	25	517
May 3, 1921	665	40	705	548	64	612
Average	457	70	527	306	49	355
Second:						
Apr. 30, 1918	437	120	557	131	66	197
May 9, 1919	746	213	959	572	86	658
May 22, 1920	761	45	806	697	116	813
May 27, 1921	714	51	765	560	93	653
Average	665	107	772	490	90	580
Third:						
June 3, 1918	538	145	683	486	73	559
May 31, 1919	758	172	930	601	118	719
June 20, 1920	702	105	807	660	149	809
July 1, 1921	652	115	767	524	145	669
Average	662	134	796	568	121	689

The most striking and perhaps the most significant differences appear in the first charting. It will be noticed that there is almost a month's difference between the earliest first charting date and the latest first charting.

In every season there was a much larger number of squares containing grasses on the burned than on the unburned areas. In 1918 there were ten times as many squares containing grasses on the burned area as on the unburned; in 1919, the difference was 88 per cent; in 1920, 26 per cent; and in 1921, 21 per cent. The decrease in the difference is no doubt due in part at least to the later charting dates in 1919, 1920, and 1921. Therefore the essential and important conclusion is that in each year there were more grasses on the burned area. This substantiates the opinion of those advocates of burning who claim that it makes green feed available at an earlier date.

Burning seems to have decreased the number of sedges while no consistent change in this respect can be observed on the unburned areas. Just why burning injured the sedges more than the grasses is not clear.

The second charting of each year showed a marked increase in the number of specimens as compared with the first charting. This is to be expected and is due to the additional growth which takes place as the season advances. The paramount factor, however, is the greater number of grasses for the burned area compared with the unburned. The increase of grass on the burned area was 233 per cent in 1918, 30 per cent in 1919, 9 per cent in 1920, and 30 per cent in 1921. The large difference in 1918 is due to the early charting date and substantiates the conclusion that growth began much earlier on the burned plot.

The number of sedges appears to have been decidedly increased by burning for two years while in the two other years a decrease was recorded.

When the third chartings were made most of the vegetative growth for the season had taken place. Nevertheless, it will be seen that in each year there were more squares containing grasses on the burned than on the unburned areas. The variation from year to year is no more than would normally be expected, since it is a well known fact that the amount of vegetation produced in any area may vary from 10 to 40 per cent. The variations shown in these tables are probably due, therefore, to seasonal conditions rather than to treatment.

Burning seems to have resulted in a decrease in the number of sedges, as they are decidedly fewer in the last two years of the experiment than in the first and second years. The number has increased somewhat on the unburned section. Since the number to begin with was less on the unburned areas, the effect of burning on these plants is not entirely clear. In this pasture, sedges are not common enough to be an important factor and hence no great importance can be attached to the changes so far recorded.

In order to give an idea of the changes in composition that are taking place on the two plots, Table III, which shows the species of grasses and sedges and their abundance in 1918 and in 1921, has been prepared.

TABLE III.—*Average composition of all quadrats on burned and unburned areas in 1918 and in 1921 (third charting)*

Species.	Burned area.		Unburned area.	
	June 3, 1918.	July 1, 1921.	June 3, 1918.	July 1, 1921.
	Squares.	*Squares.*	*Squares.*	*Squares.*
Big bluestem	165	138	133	232
Little bluestem	265	394	308	122
Side oats grama	88	12	40	11
Kentucky bluegrass	15	42	0	135
Others [1]	5	66	5	24
Sedges [2]	145	115	73	145
Total grasses	538	652	486	524

[1] Principally *Sporobolus heterolepsis* Gray.
[2] Principally *Carex pennsylvanica* Lam. and *Carex meadii* Dewey.

The most marked effect of burning has been to decrease the amount of big bluestem and increase the amount of little bluestem. The decrease in big bluestem is especially noteworthy in view of its tendency to increase on the unburned plot. This change is very noticeable when the plots are examined. There has been an increase of 48 per cent in little bluestem on the burned plot and a decrease of 60 per cent on the unburned plot. Side oats grama decreased in both cases but the decrease is much greater on the unburned plots. No Kentucky bluegrass was recorded on the unburned plots in 1918 but in 1921 there were 135 squares containing this grass. On the burned plot there were 15 squares in 1918, but only 42 in 1921, a very small increase as compared with the unburned plots. Chief among the grasses listed as "others" is *Sporobolus heterolepis*. The amount of this grass present in the burned area is quite noticeable and it appears to be increasing.

It is probable that those grasses which start earliest in the spring are injured most. The injury by burning no doubt kills some plants and

weakens others so that adverse conditions later in the season may cause their death.

It would seem from the above that the effects of burning will depend somewhat upon the composition of the pasture. A big bluestem pasture, or a Kentucky bluegrass pasture, would apparently be injured unless these grasses are replaced by equally or more desirable grasses. In many cases this, no doubt, is what would happen. There are undoubtedly other instances where they would be replaced by much less desirable species, in which instance burning would result in injury.

EFFECT OF BURNING ON WEEDS

The number and kind of weeds present was also recorded at each charting. This compilation, however, was by specimens and not by squares, as was done with grasses. The principal object was to determine whether burning retards or increases the spread of weeds. If there is a steady increase, it can be assumed that retrogression is taking place, while if no increases occur it may be assumed that the grasses are holding their own. It should be noted that there is always a considerable variation in the number of weeds present on any given area from year to year. Weeds react very quickly to favorable or unfavorable growing conditions and this should be kept in mind when analyzing the data.

The principal weeds found in the order of their importance are prairie sage (*Artemesia gnaphaloides* Nutt.), dwarf horseweed (*Erigeron divaricatus* Michx.), annual ragweed (*Ambrosia artemisiifolia* L.), whorled milkweed (*Ascelepias verticellata* L.), perennial ragweed (*Ambrosia psilostachya* D. C.), daisy fleabane (*Erigeron ramosus* Watt.), and prairie cat's foot (*Antennaria campestris* Rydberg).

Table IV shows the total number of weeds of all kinds present in all of the quadrats on the unburned and burned areas for each year.

TABLE IV.—*Number of weed specimens present on the burned and unburned areas from 1918 to 1921*

First charting.			Second charting.			Third charting.		
Date.	Burned.	Unburned.	Date.	Burned.	Unburned.	Date.	Burned.	Unburned.
Apr. 9, 1918	None.	None.	Apr. 30, 1918	None.	None.	June 3, 1918	5	1
Apr. 17, 1919	55	23	May 9, 1919	85	53	May 31, 1919	80	92
May 5, 1920	45	38	May 22, 1920	47	60	June 20, 1920	37	41
May 3, 1921	31	48	May 27, 1921	41	61	July 1, 1921	29	56

When the first and second chartings were made in 1918 no weeds were found on either the burned or unburned areas. The third charting showed five specimens on the burned area and one on the unburned. These numbers are so small that but little importance can be attached to them except to say that the plots were almost free of weeds.

In 1919, there was a decided increase in the number of weeds. When the first charting was made, there were 55 specimens of weeds on the burned and 23 specimens on the unburned area. On the second date, there were 85 and 53 specimens, respectively, and on the third date 80 and 92, respectively.

The larger number of weeds on the unburned area in the third period may have been due to the later start of the vegetation on these plots. It may be concluded from these data that the treatment of the plots had nothing to do with the increase over 1918. If the burning was responsible for these changes, there would have been a greater difference in the number of weeds on the burned and unburned areas.

In 1920, weeds were generally less abundant than in the preceding year and there were no consistent differences due to the treatment of the plots. The 1921 burning reduced the number of weeds somewhat, the differences in each case apparently being significant.

The data seem to show beyond doubt that burning does not favor the growth of weeds but that on the contrary there is a tendency towards a decrease in their number on the burned plots as compared with the unburned plots.

THE EFFECT OF BURNING ON SOIL TEMPERATURES

The principal object of recording the soil temperature was to determine if burning resulted in a warmer soil early in the spring, and if so, whether this difference could be correlated with the growth of vegetation. The thermograph bulbs were placed at depths of one and three inches respectively, because the majority of roots are found at about the latter depth and because germinating seed is ordinarily found at a depth of one inch or less.

The thermographs were usually started immediately after burning and they were all kept running until June 10. In 1921, they were started before burning in order to see if the heat generated by the burning vegetation penetrated the soil. The records show that no rise in temperatures took place in either the one-inch or three-inch bulbs, indicating that the heat of combustion is confined entirely to the immediate ground surface. It seems, therefore, that unless there is considerable accumulation of vegetation the fire does not heat the soil sufficiently to cause material damage. Usually most plants are merely scorched and it is probable that weeds and grass seeds which are lightly covered escape injury entirely. Moreover if the burning is done soon after a rain, as was done in these experiments, the moisture in the ground will aid in protecting the seeds.

The maximum and minimum temperatures were assumed to be the most significant temperatures in relation to vegetation and are the only ones considered in this paper. The mean maximum and mean minimum for each year are presented in Table V which follows. Figures 5, 6, 7 and 8 have been prepared to show the change in temperature with the advance of the season for each year.

TABLE V.—*Mean maximum and minimum temperatures at a depth of one inch on burned and unburned plots (1918 to 1921)*

Year.	Mean maximum temperatures.			Mean minimum temperatures.		
	Burned.	Unburned.	Difference.	Burned.	Unburned.	Difference.
1918	81.7	67.2	+14.5	50.2	50.0	−0.4
1919	71.6	67.1	+ 4.5	54.7	51.9	+2.8
1920	88.9	70.3	+18.6	60.7	53.3	+7.4
1921	77.0	66.4	+10.6	49.6	42.8	+6.8
Average	79.8	67.7	+12.1	53.8	49.7	+4.1

TABLE VI.—*Mean maximum and minimum temperatures at a depth of three inches on burned and unburned plots (1918 to 1921)*

Year.	Mean maximum temperatures.			Mean minimum temperatures.		
	Burned.	Unburned.	Difference.	Burned.	Unburned.	Difference.
1918	69.9	61.0	+8.9	53.4	50.4	+3.0
1919	65.8	62.1	+3.7	54.7	51.9	+2.8
1920	72.6	72.8	−.2	61.8	55.5	+6.3
1921	66.6	64.8	+1.8	52.1	47.3	+4.8
Average	68.7	65.1	+3.6	55.5	51.3	+4.2

In every year, as shown in Table V, the mean maximum temperature of the soil at a depth of one inch, averaged materially higher on the burned plot. This difference ranged from 4.5° F. in 1919, to 18.6° in 1920. The average difference was 12.1°. A higher mean minimum temperature was also recorded for the burned plots except in 1918, when the difference was negligible. The average difference it will be noted is 4.1°.

Similar, although less marked differences were recorded at a depth of three inches (Table VI), where the average difference in the mean maximum temperature was 3.6°F. and in the mean minimum temperature 4.2°. These results show beyond a reasonable doubt that the soil on the burned plots absorbed more heat, which no doubt explains the earlier growth of vegetation previously pointed out.

As shown in figures 1, 2, 3, and 4, the burned plots were consistently warmer throughout the season than the unburned plots, as measured by the weekly mean maximum temperatures at a depth of one inch, with a few exceptions. The mean minimum temperatures were also in favor of the burned plots. It appears that the differences in early growth of vegetation may easily be accounted for by these differences in temperature.

EFFECT OF BURNING ON THE YIELD OF HAY

The yield of hay was usually determined about September 10, each year, by harvesting several areas, usually ten in number. These areas, each one-ten-thousandth of an acre, were selected by means of the iron hoop previously mentioned.

The results expressed in pounds of dry matter per acre are shown for each year in Table IX.

TABLE IX.—*Showing the yields of cured hay on burned and unburned plots (1918 to 1921)*

Year.	Pounds per acre.		
	Burned.	Unburned.	Difference (in favor of burned).
1918	3,075	4,623	−1,548
1919	3,500	3,633	−133
1920	1,730	1,774	−44
1921	3,156	2,973	+183
Average	2,865	3,251	−386

The large difference in the yield between the burned and unburned areas in 1918 is difficult to explain, since the quadrat chartings up until June 3 indicated considerably more shoots on the burned areas than on the unburned. This apparently should mean higher yields. It seems probable that in obtaining the cuttings a portion of the dried material remaining from 1917 was included.

There was a slight difference in yield in 1919 and in 1920, in favor of the unburned plots, but it is too small to be considered of much impor-

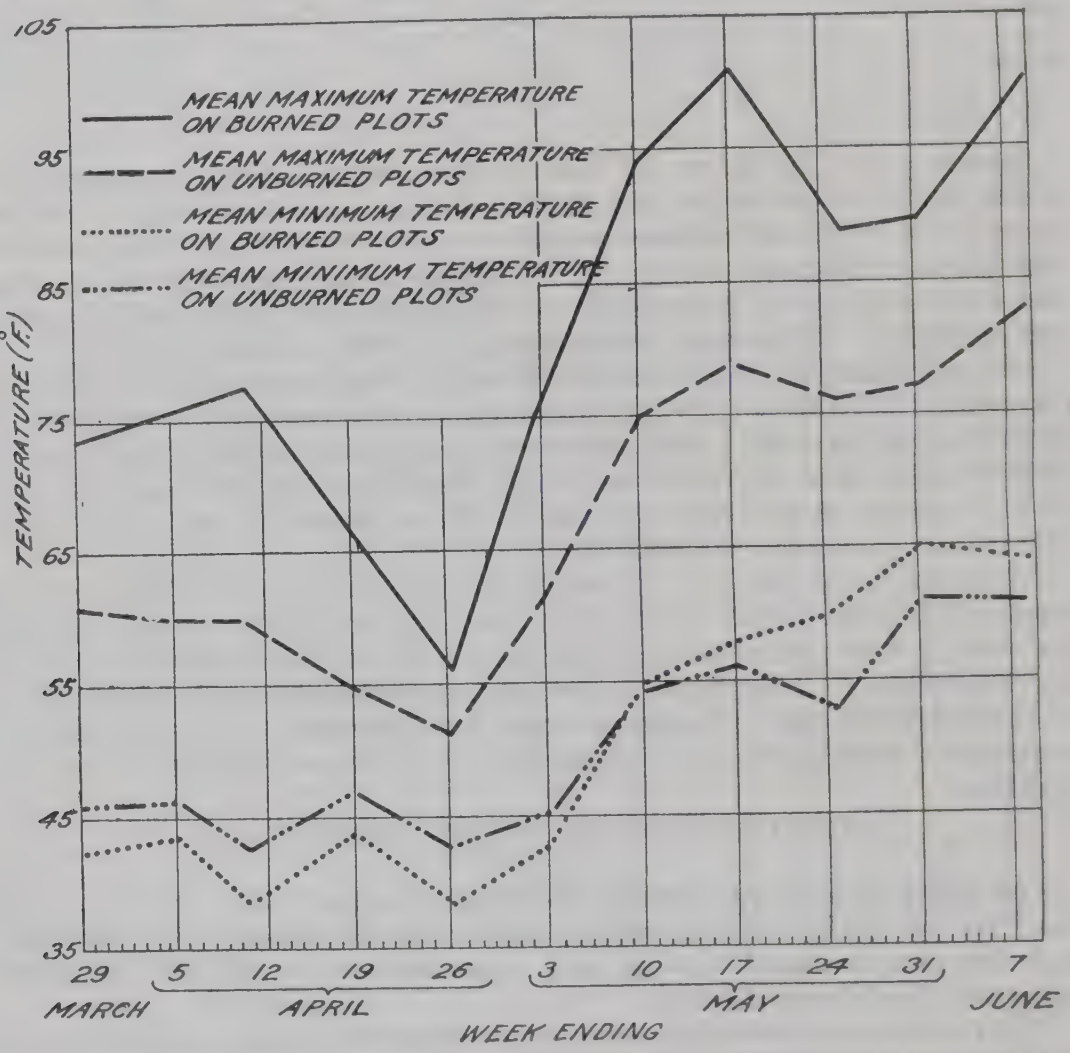


FIG. 1.—Graph showing weekly mean maximum and minimum temperatures in 1918 at a depth of 1 inch under the surface on burned and unburned plots.

tance. Conditions were unfavorable in 1920, and the grasses showed a poor growth.

In 1921 the burned area produced the higher yield, the difference being 183 pounds per acre. Conditions were subnormal in the early season. There was not much rainfall early in the spring and cold weather persisted until June. Later on, however, good rains fell and grass showed excellent growth. The average difference of 386 pounds per acre in favor of the unburned is largely due to the 1918 results. Considering the data for 1919, 1920, and 1921 only, it will be seen that the amounts of hay produced on the two areas are practically equal.

The general opinion among those who are opposed to burning is that much more forage is produced on areas not burned. It is believed that this conclusion is erroneous, since the experiments here reported have

failed to show, so far, that much higher yields are actually produced on unburned areas. Considering the small difference in yield and the marked difference in the number of desirable grasses in favor of the burned plot, it can not be concluded that burning has caused any injury to the pasture.

The last word regarding burning has yet to be written. The results secured, as these experiments show, will likely depend not only on seasonal conditions but also upon the kind of grasses and other vegetation present, and more extensive experiments with a wide variety of conditions will be necessary to determine just what may in general be expected of burning.

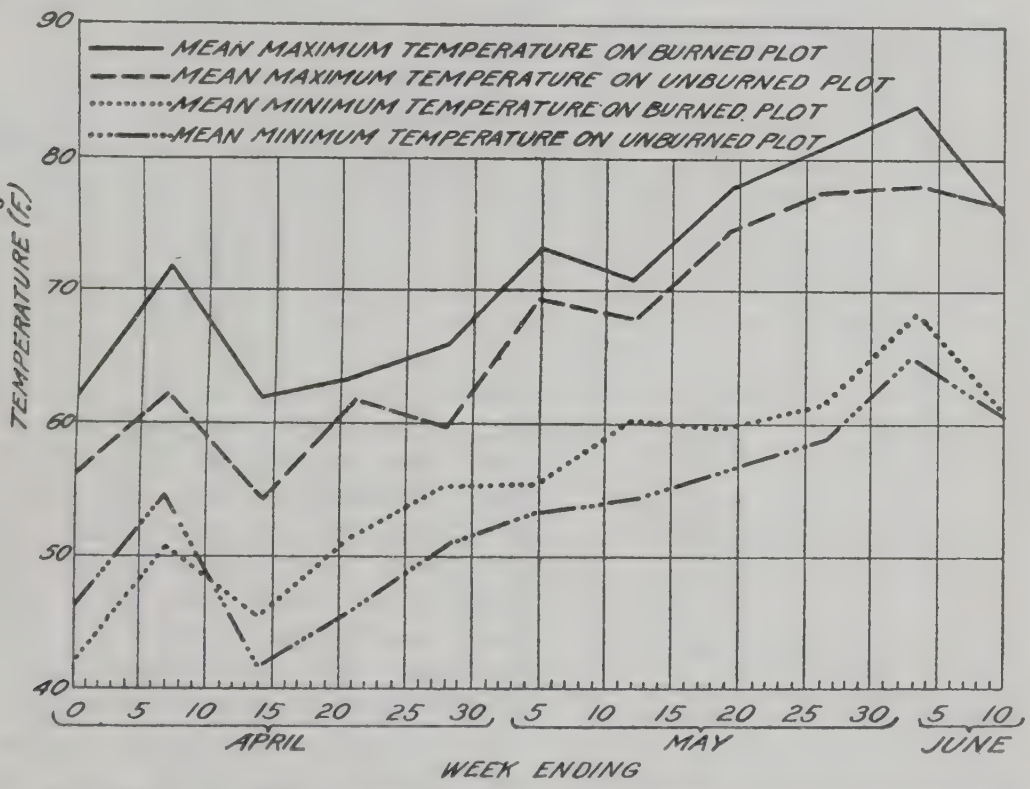


FIG. 2.—Graph showing weekly mean maximum and minimum temperatures in 1919 at a depth of 1 inch under the surface on burned and unburned plots.

SUMMARY

(1) The data presented in this paper have been obtained in connection with pasture investigations conducted by the Kansas Agricultural Experiment Station. The experiments discussed were designed to study the effect of burning on the vegetation in pastures.

(2) Areas were burned early each year for comparison with similar areas not burned. A number of small quadrats in each area were charted three times between the time of burning and July 1 each year. The results show that in the early part of the season there was considerably more growth of grasses on burned areas than on those unburned, thereby substantiating the popular opinion that burning causes growth to start earlier in the spring. In the second charting there was always more vegetation on the burned areas but the difference was not so great as in the first charting. The third charting was done when the greater part of the season's growth had been made. The differences found in the third charting were slight, showing that as the season advanced the vegetation on the unburned sections tended to catch up with that on the burned areas.

(3) Burning did not decrease the total number of grass plants, as is generally thought to be the case. The data show that at the end of four years there were 21 per cent more squares containing grass plants on the burned area than there were when the experiment started. On the other hand, the increase in the unburned plot was only 7 per cent. These data, while not final, indicate that the evil effects of burning, if any, are not always apparent in the first four years.

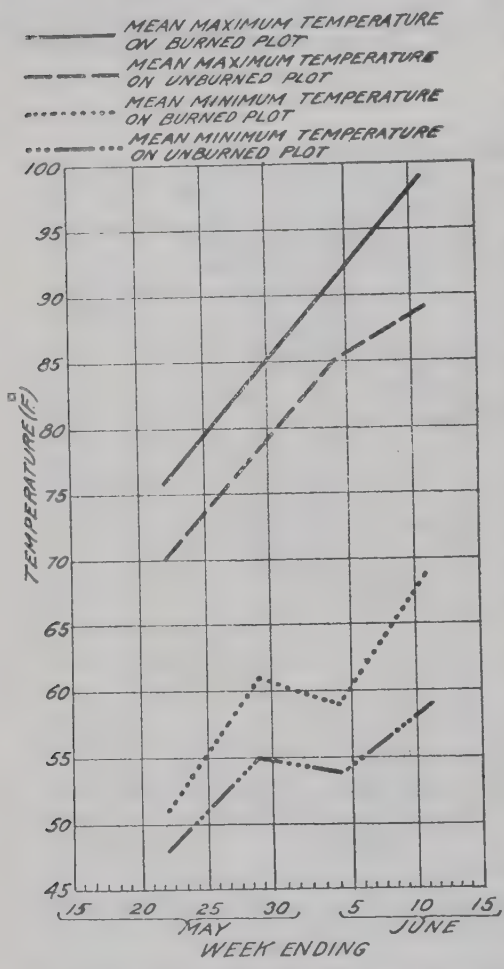


FIG. 3.—Graph showing weekly mean maximum and minimum temperatures in 1920 at a depth of 1 inch under the surface on burned and unburned plots.

(4) There was a decrease during the four years in the number of sedges on the burned area, while on the unburned plot a tendency to increase was observed.

(5) Burning caused a change in the composition of the grass type on the experimental area. Big bluestem (*Andropogon furcatus*) decreased on the burned plot, while on the unburned plot it showed a decided increase. On the other hand, burning increased the number of plants of little bluestem (*Andropogon scoparius*), although the number decreased on the unburned plot. Kentucky bluegrass increased much more rapidly on the unburned area. Bluegrass starts very early in the spring, and it appears that it may have been more easily injured than other grasses which start late. Many plants were green when the burning was done. Side oats grama decreased on both burned and unburned plots.

(6) In 1918, when the experiments were started, both areas were practically free of weeds. In 1919 a great many were found on both burned and unburned plots. From this date on there was a decrease each year in the number of weeds on the burned plot, while on the unburned one their number increased.

(7) Mean maximum soil temperatures at a depth of 1 inch averaged 12.1° F. higher on the burned plot. The mean minimum temperatures were also higher by 4.1° on the burned plot. Similarly, at a depth of 3 inches mean maximum and minimum temperatures were 3.6° and 4.2° higher, respectively, on the burned plot. These data may explain why growth starts earlier when the old vegetation has been removed by burning.

(8) The vegetation from each plot was cut about September 10 each year. The average yield was slightly greater on the unburned area. The difference, however, was slight, except during one season, and in another season it was in favor of the burned plot.

(9) The conclusion is that studies so far conducted have failed to show that burning is injurious. More extensive experiments with different types of vegetation and for a longer period of time must be conducted before final conclusions regarding the effects of burning can be arrived at.

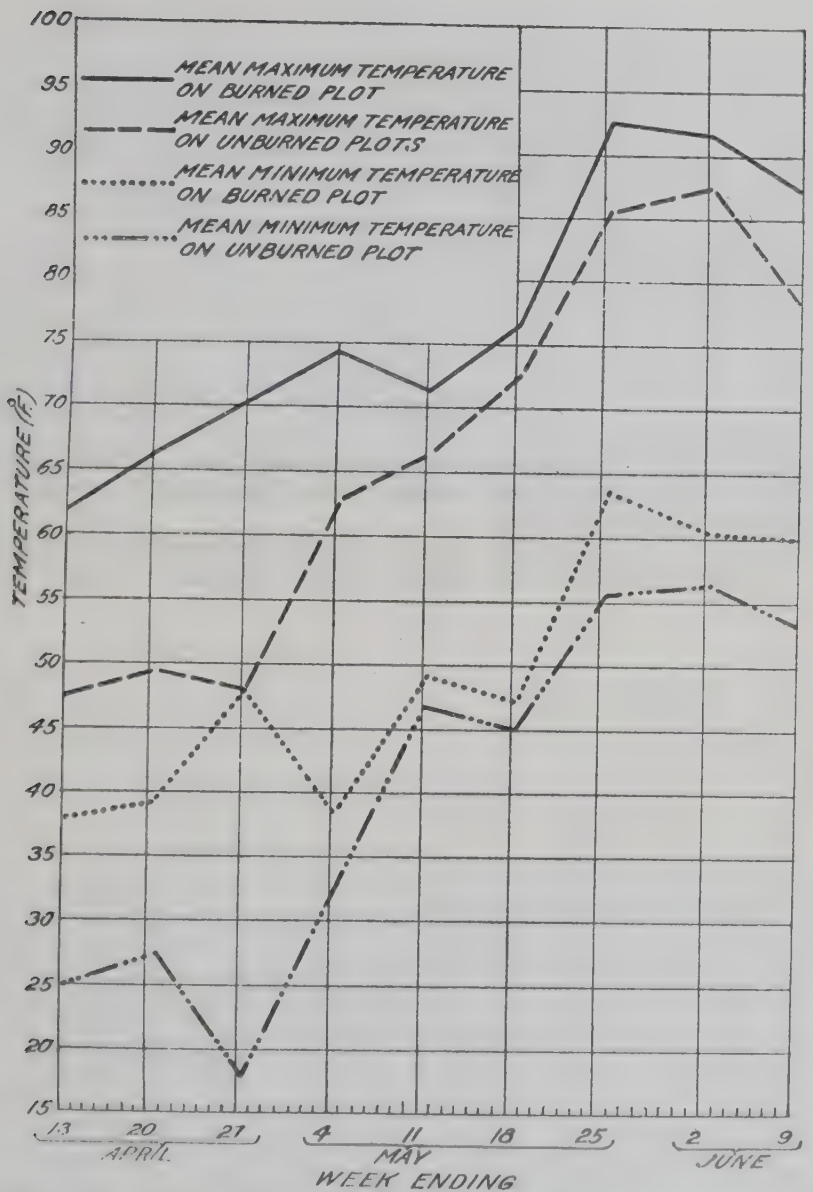


FIG. 4.—Graph showing weekly mean maximum and minimum temperatures in 1921 at a depth of 1inch under the surface on burned and unburned plots.

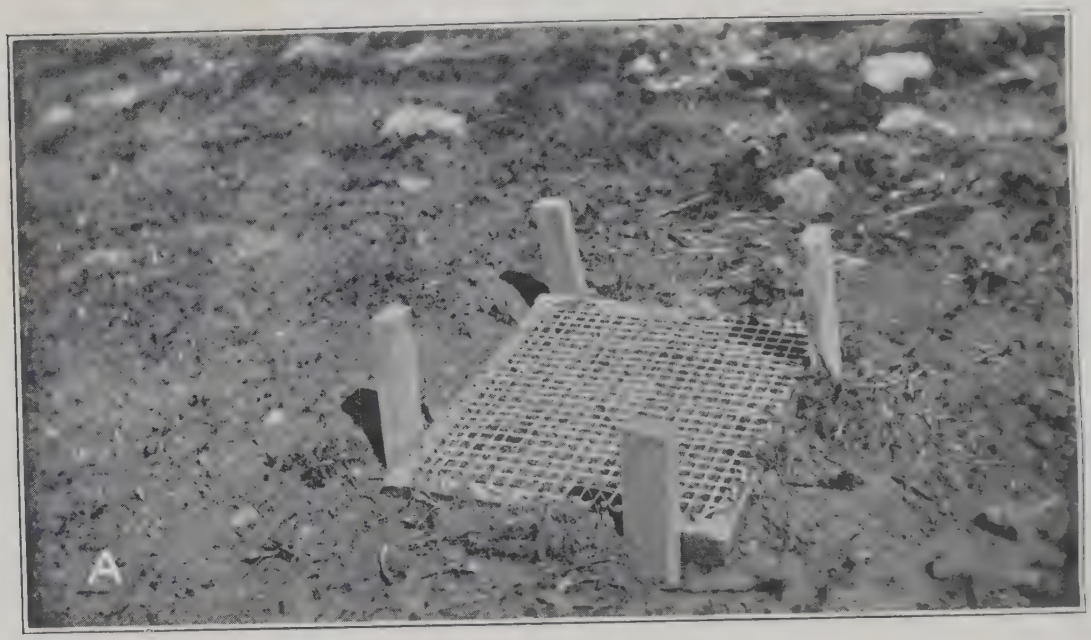
PLATE I

A.—General view of the plot which was burned each year. The plot in the foreground is the one which was never burned. The illustration shows how close this latter area was cut to imitate grazing.

B.—Showing the vegetation on the unburned plot after cutting to imitate grazing. The portion on the right shows the original stand before the cutting was done.

A

B


A

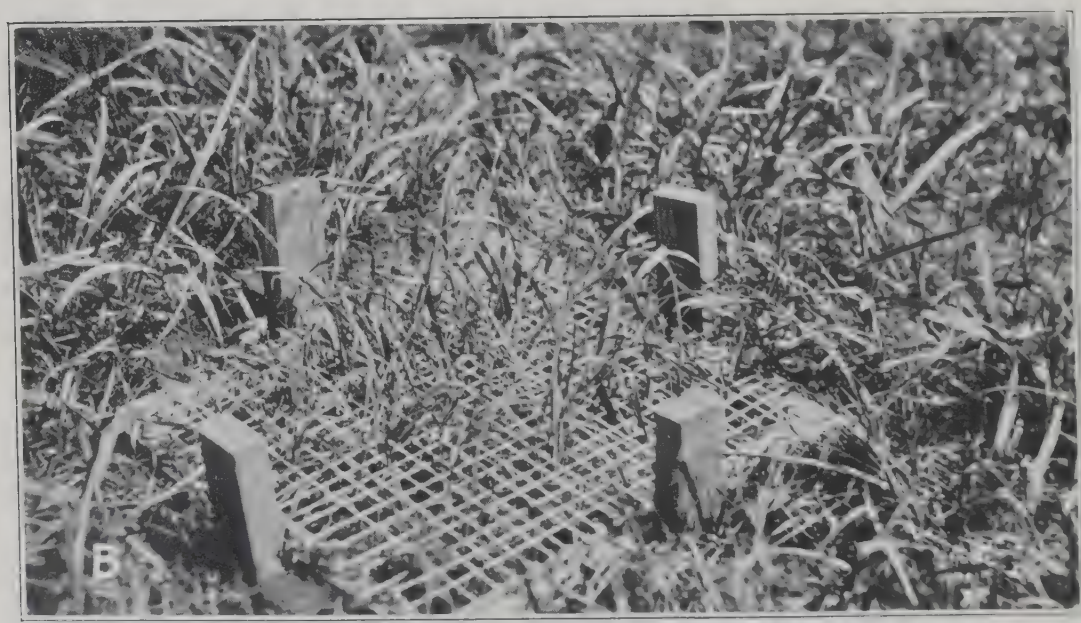

B

PLATE 2

A.—One of the 10 by 10 inch quadrats permanently staked and ready for the season's work. The interstices are one-half inch square. The area upon which this quadrat is located had just been burned when this photograph was taken.

B.—A view of the same quadrat as is shown in A, taken a few weeks after burning. Note the clean, vigorous growth and the absence of last year's litter.

CONTROL OF LETTUCE DROP BY THE USE OF FORMALDEHYDE[1]

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INTRODUCTION

The growing of head lettuce under glass has been developed to a very high degree in the Boston market garden district and other parts of Massachusetts. It is usually the main greenhouse crop, and not uncommonly the market gardener grows 4 to 6 acres under glass. In many of these houses the soil has not been changed for 20 years or more. As a result it has become so thoroughly infested with *Sclerotinia libertiana* Fckl., the fungus which causes drop, that the loss to the crop is sometimes enormous.

Investigations on this disease were begun by Stone and Smith (*7, p. 3*)[3] of the Massachusetts Agricultural Experiment Station as long ago as 1895. In the course of their work they devised a method of soil sterilization by the use of steam, and since 1900 this has been in more or less general use for the control of drop. The method has some serious disadvantages, however, which led in 1914 to the reopening of the investigation by the department of botany of the same station.

REVIEW OF LITERATURE

There are few experimental data available dealing specifically with the control of *S. libertiana* by the use of formaldehyde. Smith (*5*) states that the mycelium of *S. libertiana*, growing on pieces of lemon crates, was killed by immersing it for 1, 5, and 10 minute periods in a 5 per cent formaldehyde solution. Treating the same fungus with formaldehyde as it occurs naturally in lettuce beds, Stevens (*6*) and Reddick (*8, p. 200*) report negative results, whereas Clinton's (*4*) results were of a positive nature. Since the completion of this work Beach (*2*) reports slight gains with the use of formaldehyde on out-of-doors lettuce beds. Also, *New Jersey Agriculture* (*1*) reports similar results with the use of formaldehyde on out-of-doors seed beds.

Although the literature contains conflicting reports on the efficacy of formaldehyde used as a soil disinfectant, our own preliminary tests indicated that the material might have considerable value in the control of lettuce drop. Consequently the work was concentrated on the use of formaldehyde in this connection, and extensive laboratory and greenhouse tests have been made. The work has included treatment of large commercial greenhouses on a practical basis.

[1] Accepted for publication Jan. 16, 1922. Published with the approval of the director, Massachusetts Agricultural Experiment Station.

[2] The writer is greatly indebted to Prof. A. Vincent Osmun, head of the Department of Botany of this Station for his many helpful suggestions during the progres of this study.

[3] Reference is made by number (italic) to "Literature cited," p. 654.

LABORATORY TESTS OF THE EFFECT OF FORMALDEHYDE ON SCLEROTIA AND MYCELIUM

TREATMENT OF SCLEROTIA

Some very interesting data were obtained by treating sclerotia of *S. libertiana* in vitro for different periods of time with different concentrations of formaldehyde. Films of air covered portions of the surface of the sclerotia when first placed in the solution. This difficulty was overcome by vigorous agitation in distilled water for one-half hour immediately preceding treatment. Sclerotia specified as small were 1 mm. or less in diameter; those designated as large were over 1 mm. in diameter.

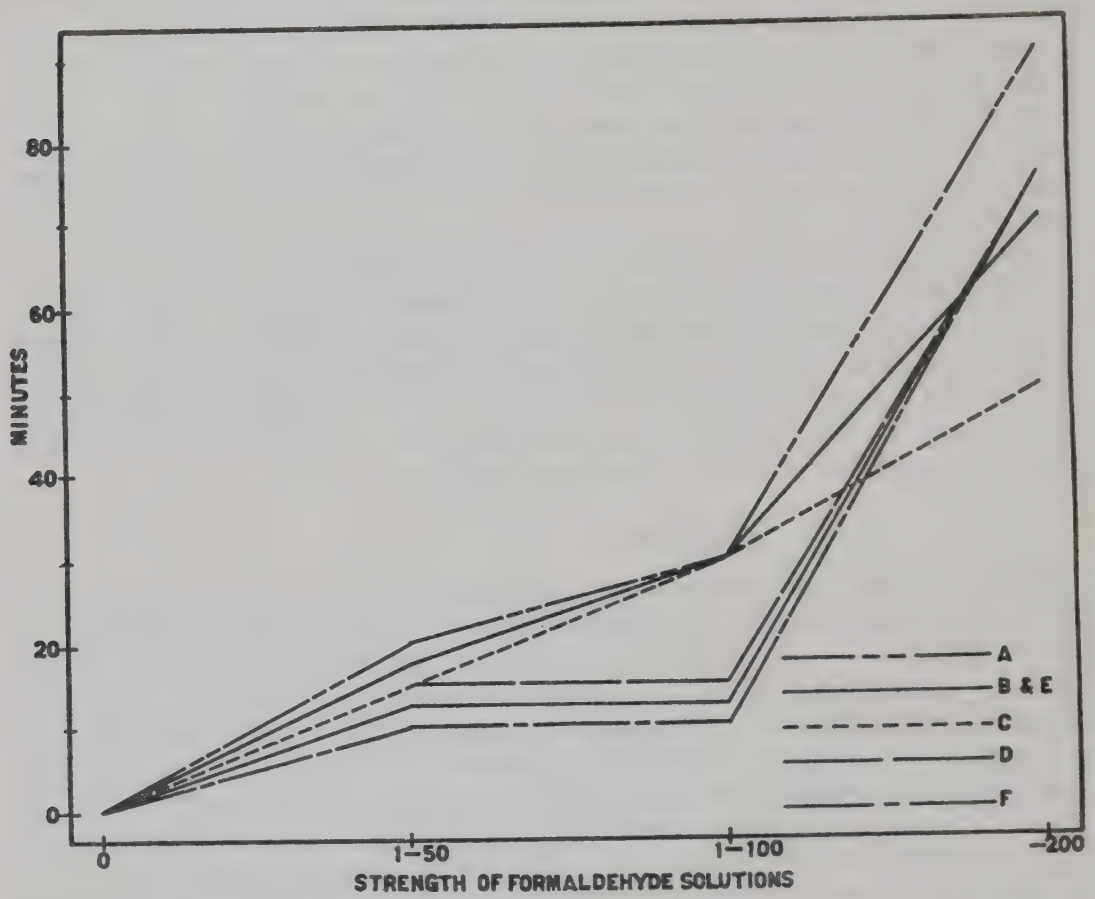

FIG. 1.—Graph made from Table I, showing the maximum and minimum time required in minutes to kill large and small sclerotia in 1–50, 1–100, and 1–200 formaldehyde solutions. Curve A shows the maximum time required to kill large sclerotia with these solutions; curve C shows the minimum time required to kill sclerotia; and curve B is an average of the total treatments made on large sclerotia. Curve D represents the maximum time required to kill small sclerotia with these solutions; curve F shows the minimum time required; and curve E is an average of the results obtained with small sclerotia.

Sclerotia (Tables I and III) were taken from diseased lettuce plants after they had "dropped" in the bed. The sclerotia were grouped into lots of nine each according to size . After shaking in distilled water they were transferred to 400-cc. air-tight glass jars containing formaldehyde solutions of the desired strengths,[4] allowed to remain for different periods, after which they were removed under aseptic conditions and thoroughly washed three times in sterile distilled water. From this they were transferred to nutrient agar plates and the results of growth recorded (Tables I and III). Under these conditions, in a 1–50 solution the maximum time

[4] 1–50, 1–100, and 1–200 formaldehyde solutions were made by using one part of 37.3 per cent formaldehyde to 49, 99, and 199 parts, respectively, of distilled water.

required to kill small sclerotia was 15 minutes (Table I, treatments 1 and 3), while all large sclerotia were killed in 20 minutes (Table I, treatments 2 and 4). In a 1–100 solution the maximum time required to kill small sclerotia was 15 minutes (Table III; treatments 1, 3, and 8), whereas all large sclerotia were killed in 30 minutes (Table III, treatments 2, 4, 7, and 9). In a 1–200 solution the maximum time required to kill small sclerotia was 75 minutes (Table I, treatment 5), while all large sclerotia were killed in 90 minutes (Table I, treatments 6 and 7). In each concentration the small sclerotia were killed in less time than the large. Also, results show (fig. 1) that the longest time required to kill sclerotia of any size is inversely proportional to the strength of the solution. Furthermore, it should be noted (fig. 1) that a 1–100 formaldehyde solution is almost as effective as a 1–50 solution but that a 1–200 solution is much less effective than the stronger solutions.

Sclerotia of lots 1, 2, 3, 4, and 7 (Table I) were taken from plants that showed drop symptoms 10 days before the sclerotia were removed; lots 5 and 6 were taken 12 days after the first symptoms of drop were noticed on the plants.

TABLE I.—*Effect of formaldehyde on sclerotia*

Treatment.	Size of sclerotia, lots of 9.	Number viable in control, untreated.	Number viable after treatment.												
			3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	25 min.	30 min.	50 min.	75 min.	90 min.	100 min.	120 min.
1. Formaldehyde, 1–50.	Small...	6	7	5	3	1	0	...	...	...	...	...	...	...	...
2. Formaldehyde, 1–50.	Large....	9	7	7	6	5	3	0	...	...	...	...	...	...	...
3. Formaldehyde, 1–50.	Small...	8	...	6	...	0	0	0	0	0	0	0	...	...	...
4. Formaldehyde, 1–50.	Large....	7	...	...	...	5	0	0	0	0	0	0	...	...	...
5. Formaldehyde, 1–200	Small...	9	...	...	...	9	...	9	...	9	2	0	0	...	0
6. Formaldehyde, 1–200	Large....	9	...	...	...	...	...	7	...	7	0	0	0	...	0
7. Formaldehyde, 1–200	Large....	8	...	3	...	3	7	4	...	8	9	2	0	...	...

TREATMENT OF MYCELIUM

The white aerial mycelium of pure cultures or of diseased lettuce plants was thoroughly moistened by transferring for a few minutes to sterile distilled water. It was then removed and treated with the desired strength of formaldehyde solution for different periods of time. At the end of the exposure the mycelium was removed aseptically, washed in sterile distilled water, and transferred to nutrient agar plates. The results are recorded in Table II.

Mycelium from pure culture was killed in 5 to 10 minutes in a 1–50 or a 1–100 formaldehyde solution, and in 15 to 20 minutes in a 1–200 solution. Mycelium from diseased lettuce plants was killed in 10 to 15 minutes in a 1–50 or a 1–100 formaldehyde solution (Table II). The mycelium of pure cultures was killed in less time than mycelium from diseased lettuce plants.

The curve of figure 2 shows that the 1–50 and the 1–100 formaldehyde solutions are equally efficient in killing the mycelium of *S. libertiana* and that the 1–200 solution is decidedly less effective than the other two. It is interesting to note that this held true in most cases throughout these experiments.

TABLE II.—*Effect of formaldehyde on mycelium*[1]

Treatment.	Number viable in control, untreated.	Number viable after treatment.						
		3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	30 min.
1. Formaldehyde, 1–50.....	9	9	8	7	2	0	0	0
2. Formaldehyde, 1–50.....	9	.....	1	.....	0	0	0	0
3. Formaldehyde, 1–50.....	9	.....	0	.....	0	0	0	0
4. Formaldehyde, 1–100.....	9	8	8	4	5	0	0	0
5. Formaldehyde, 1–100.....	9	.....	9	.....	0	0	0	0
6. Formaldehyde, 1–100.....	9	.....	4	.....	0	0	0	0
7. Formaldehyde, 1–200.....	9	.....	9	.....	9	4	0	0
8. Formaldehyde, 1–200.....	9	.....	9	.....	9	7	0	0

[1] The mycelium of treatments 1 and 4 was taken from diseased lettuce plants; all others from the surface of 8-day-old agar cultures. Nine lots of mycelium used in each case.

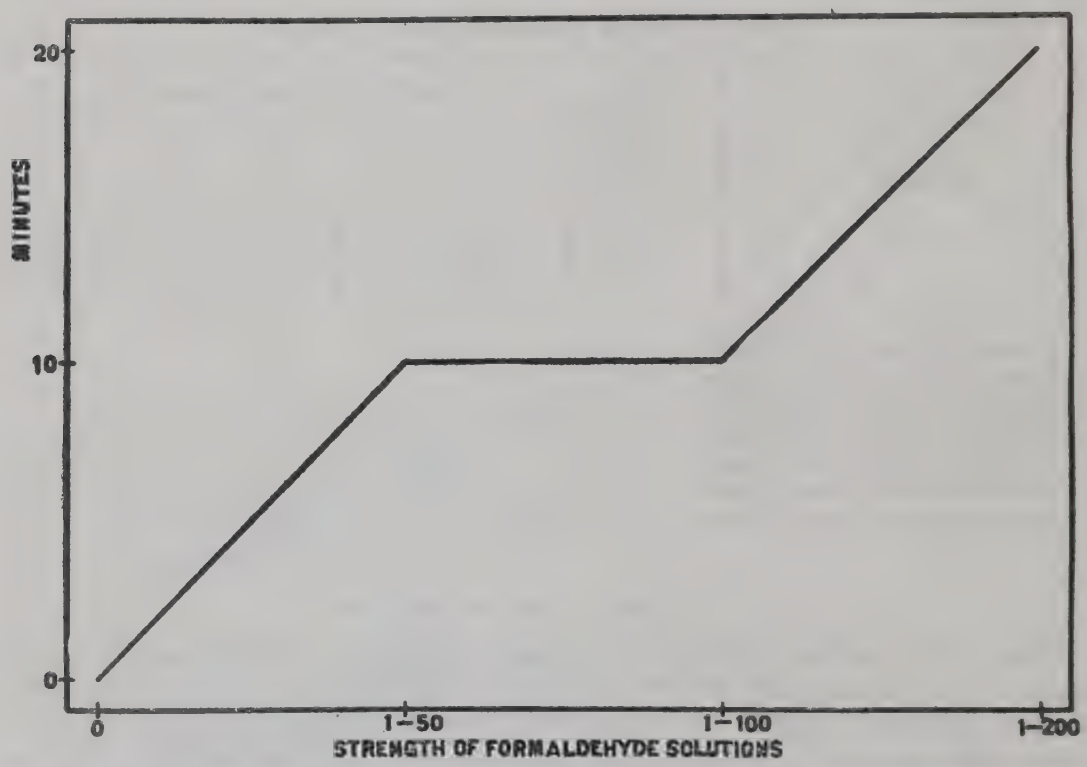

FIG. 2.—Graph showing the maximum time in minutes required to kill the mycelium of *S. libertiana* in 1–50, 1–100, and 1–200 formaldehyde solutions.

EFFECT OF ASPIRATING SCLEROTIA ON SUBSEQUENT TREATMENT WITH FORMALDEHYDE

To test the effect which surface and intercellular air might have on the action of formaldehyde on sclerotia, a number of sclerotia were aspirated in water in an aspirating bottle for ½ hour. Subsequently they were treated with a 1–100 formaldehyde solution, washed aseptically, and plated on nutrient agar. A comparison with the untreated controls shows that the process failed to shorten the time in which the sclerotia were killed. (Table III, treatments 6 and 7.)

Sclerotia of lot 2 (Table III) were taken 12 days after the first symptoms of drop were noticed on the plants; lots 1 and 3 were taken 14 and 18 days, respectively, after the first symptoms of drop were noticed on the plants. Lots 6 and 7 were aspirated for ½ hour previous to treatment, while lots 8 and 9 were kept moist in soil for 18 days previous to treatment. Lots 10 and 11 were air-dried for 18 days previous to treatment, and lots 4 and 5 for 16 and 35 days, respectively.

TABLE III.—*Effect of formaldehyde on sclerotia*

Treatment.	Size of sclerotia, lots of 9.	Number viable in control, untreated.	Number viable after treatment.													
			3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	25 min.	30 min.	50 min.	75 min.	90 min.	100 min.	120 min.	261 min.
1. Formaldehyde, 1–100..	Small...	8	..	5	..	0	0	0	...	0	0	0	..	..	..	..
2. Formaldehyde, 1–100..	Large...	9	..	9	..	9	7	4	...	0	0	0	..	..	..	..
3. Formaldehyde, 1–100..	Small...	5	..	6	..	2	0	0	0	0	0	0	..	..	..	..
4. Formaldehyde, 1–100..	Large...	7	..	4	..	6	2	1	...	0	0	0	..	..	..	..
5. Formaldehyde, 1–100..	Large...	4	..	...	..	5	4	5	2	2	0	1	..	..	..	..
6. Formaldehyde, 1–100..	Small...	7	..	6	..	9	4	1	0	0	...	...	..	..	..	..
7. Formaldehyde, 1–100..	Large...	6	..	...	..	7	7	1	4	...	0	...	..	..	..	..
8. Formaldehyde, 1–100..	Small...	7	..	0	..	0	0	0	...	0	0	0	..	..	..	..
9. Formaldehyde, 1–100..	Large...	8	..	0	..	0	0	0	...	0	0	0	..	..	..	..
10. Formaldehyde, 1–100..	Small...	7	..	6	..	4	2	0	...	0	0	0	..	0	..	..
11. Formaldehyde, 1–100..	Large...	8	..	9	..	9	7	4	...	3	0	0	0	0	..	..

RELATION OF MOISTURE TO THE ACTION OF FORMALDEHYDE ON SCLEROTIA

Preliminary work showed that the time required to kill the sclerotia of this fungus in different concentrations of formaldehyde solution is correlated with desiccation. For further study of this phase one lot each of large and small sclerotia was kept in the laboratory in a dry bottle plugged with cotton, while similar lots were buried in moist loam soil. At the end of 18 days the four lots were removed and treated with 1–100 formaldehyde solution. Both small and large sclerotia kept under moist conditions described above were killed in less than 5 minutes (Table III, treatments 8 and 9). Small dry sclerotia were killed in 15 to 20 minutes (Table III, treatment 10), while large dry sclerotia were killed in 30 to 50 minutes (Table III, treatment 11). When the period of air-drying was extended to 35 days, large sclerotia were not killed by an exposure of 75 minutes (Table III, treatment 5). In all the experiments where sclerotia were stored under moist conditions the formaldehyde solutions were more effective, which, from the practical viewpoint, emphasizes the need of keeping the soil thoroughly moist from 5 to 10 days before treatment.

INHIBITORY EFFECT OF FORMALDEHYDE ON CULTURAL GROWTH OF S. LIBERTIANA

During the progress of this study it was found that the solutions of formaldehyde greatly inhibited subsequent growth on agar of sclerotia and mycelium which were not killed during the treatment—that is, growth was delayed by the treatment. It appeared that the action of

the formaldehyde on the mycelium was to produce partial dehydration of the cells from which there was slow recovery, or in the case of the sclerotia, the formaldehyde killed the outer, but not the inner cells, in which case the inner cells developed growth slowly because of their position. To illustrate the retarding effect of formaldehyde solutions on subsequent growth of sclerotia and mycelium a brief account is given of an average treatment with each strength.

ONE-TO-FIFTY FORMALDEHYDE SOLUTION.—Sclerotia were treated in the solution, washed aseptically, and plated on nutrient agar. Results taken 6 days later showed that sclerotia kept in the solution for 10 minutes made an average colony growth of 8 mm. in diameter, while the sclerotia kept in a solution of the same concentration longer than 10

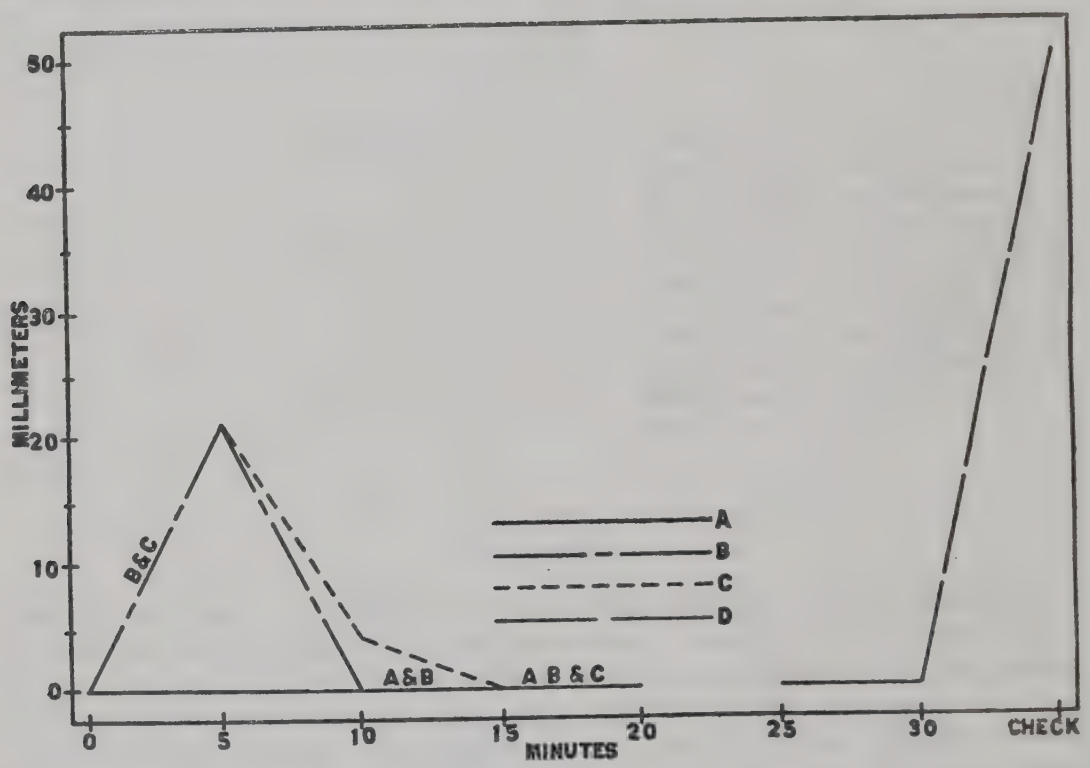

FIG. 3.—Graph showing the inhibitory effect of different concentrations of formaldehyde on the subsequent growth of mycelium treated and transferred to agar plates. Curve A is a straight line and shows that in a 1–50 solution growth was not evident after the shortest exposures; curve B shows similar results with a 1–100 solution; curve C with a 1–200 solution. There was no 25-minute exposure, hence, the skip in line A, B, C at this period. The growth of the colonies from the untreated mycelium forms the last part of each curve. All measurements taken at the end of the fourth day.

minutes were completely killed. Untreated sclerotia, cultured on the same kind of agar for 6 days, developed colonies averaging 32 mm. in diameter.

Ninety particles of mycelium from nutrient agar cultures were treated in the solution for different lengths of time, washed and plated on nutrient agar (Table II). Only 1 of the 90 particles showed growth. This one remained in the solution for 5 minutes and made a scant growth at the end of 11 days. Later the colony developed five sclerotia. The controls showed vigorous growth at the end of the first day, each colony producing from 2 to 3 dozen sclerotia.

ONE-TO-ONE-HUNDRED FORMALDEHYDE SOLUTION.—The method of procedure was the same as with the 1–50 solution. After 6 days, sclerotia kept in the solution for 10 minutes developed an average colony growth 26 mm. in diameter, while those exposed for 15 minutes produced an

average growth of 5 mm. Sclerotia exposed 20, 25, and 50 minutes showed no growth at the end of 6 days, but at the end of 21 days a few sclerotia treated for 20 and 25 minutes showed scant growth. The untreated sclerotia produced an average colony growth of 31 mm.

Nine out of 90 particles of mycelium showed growth (Table II); these remained in the solution only 5 minutes and showed no growth until the fourth day after they were transferred to the agar. The controls showed a vigorous growth at the end of the first day. Colonies from treated particles of mycelium developed sclerotia more slowly and fewer in number than those of the controls.

ONE-TO-TWO-HUNDRED FORMALDEHYDE SOLUTION.—The method of procedure was the same as with the 1–50 solution. Results taken 6 days after treatment showed that sclerotia kept in the solution for 20 minutes produced colonies averaging 17 mm. in diameter, while those exposed for 30 minutes developed colonies averaging 13 mm. Sclerotia exposed for greater periods were killed. Colony growth from the sclerotia of the untreated lot showed an average diameter of 43 mm.

Out of 90 bits of mycelium which were treated for different lengths of time, as shown in Table II, 18 were treated for 5 minutes. All of these developed with retarded growth. Eighteen were treated for 10 minutes, 14 of which were growing at the end of the fourth day and 18 at the end of the eleventh day. Of 18 treated for 15 minutes, none were growing at the end of four days, but 12 showed growth at the end of 11 days. Particles treated for 20 and 30 minutes were killed. The 18 controls showed vigorous growth at the end of the first day.

EFFECT OF FORMALDEHYDE ON SCLEROTIA AND MYCELIUM AT DIFFERENT DEPTHS IN SOIL

From the practical as well as the scientific viewpoint it is important to know to what depth sclerotia and mycelium are killed by a 1–100 formaldehyde solution applied to the surface of the soil. To determine this, sclerotia and mycelium were collected, placed in labeled copper wire baskets, and buried in a good lettuce soil at different depths (Table IV). Subsequently, the surface of the soil was treated with a 1–100 formaldehyde solution at the rate of 1 gallon to the square foot. At the end of 7 days the baskets were removed, opened, and 9 sclerotia from each depth washed in sterile distilled water and transferred to three nutrient agar plates. The particles of mycelium were treated in a similar manner. Growth was recorded at the end of the first, second, and third weeks. Final results from a large number of tests are recorded in Table IV.

Infested lettuce soils of commercial greenhouses contain sclerotia and mycelium of the causal fungus at varying depths. By the method just described it is possible to treat the organism under conditions practically the same as those in the soil of commercial houses and still recover the organism from the soil at will.

Table IV shows that with the exception of one particle buried at a depth of 16 inches all mycelium down to 20 inches was killed.

TABLE IV.—*Effect of formaldehyde on sclerotia and mycelium buried at different depths in soil*

Part of fungus treated.	Viable, surface.	Viable, 4 inches.	Viable, 8 inches.	Viable, 12 inches.	Viable, 16 inches.	Viable, 20 inches.	Moisture condition when buried.
Sclerotia from lettuce plants which showed first symptoms of drop 8 days before sclerotia were treated.	0	0	0	0	0	0	Moist.
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	0	0	0	
	0	0	0	2	0	0	
	0	0	0	1	0	0	
Sclerotia from infected lettuce plants.	0	0	1	5	3	3	Sclerotia air-dried for 15 days.
	0	0	2	0	4	0	
	0	0	0	1	0	2	
Sclerotia from pure culture 19 days.	0	0	0	2	0	2	
Sclerotia from pure culture 20 days.	0	0	0	2	8	5	
White mycelium from surface of infected lettuce plants.	0	0	0	0	1	0	Moist.
Mycelium in decomposing lettuce leaves.	0	0	0	0	0	0	

As previously stated, the moisture content of sclerotia at the time of treatment is a very important factor in killing sclerotia with formaldehyde. This is again made evident in Table IV by comparing the effect of treatment on sclerotia in which a moisture content was maintained by contact with the host plant, and on air-dried sclerotia. In eight trials where sclerotia were removed directly from the host and treated, six showed all sclerotia killed down to 20 inches. In the other two trials the treatment was effective at a maximum depth of 12 inches. Sclerotia air-dried for 15 days were killed at a depth of 4 to 8 inches. Sclerotia taken from pure culture and treated in the same manner were apparently about as resistant to the action of formaldehyde solutions in the soil as sclerotia taken from lettuce plants. Obstructions in the soil such as pebbles, rocks, dry earth, etc., may act as shields preventing the formaldehyde from reaching the fungus, which possibly explains the variations in results.

A sclerotium has been defined as "a compact mass of hyphae in dormant state." The compactness of the dormant hyphae and the thick walls of the cells makes it extremely difficult for most disinfectants to penetrate to the interior cells of a sclerotium. Although formaldehyde is very penetrating it failed to kill the sclerotial stage of the fungus as quickly as the vegetative stage under the same conditions (Table IV).

TREATMENT OF GREENHOUSE SOILS FOR THE CONTROL OF S. LIBERTIANA

Since laboratory tests with formaldehyde proved so effective against the sclerotia and mycelium of *S. libertiana* it seemed advisable to test thoroughly its value for the control of this fungus as it occurs naturally in greenhouse lettuce soils. Therefore, extensive experiments have been

conducted along these lines, both in experimental and commercial greenhouses.

In eight successive lettuce crops grown in the experimental greenhouses at Amherst, the treated plots averaged 2 per cent drop, whereas the untreated plots averaged over 50 per cent drop (Pl. 1). Several large commercial houses were treated with results equally good.

In the course of the work in was shown that the treatment in all commercial houses should begin in the lettuce seed bed and that transplanting and permanent beds also must be treated. In all of the greenhouse experiments the soil was drenched with a 1–100 formaldehyde solution applied at the rate of 1 gallon to the square foot of soil surface. No covering was used over the soil after treatment, as it was found to be impractical on a large scale and the results showed that it added nothing to the efficiency of the treatment. If at any time sterilization is not complete or a house has only a few scattered cases of drop the location of the dropped plants can be marked with pot markers as the disease appears and the roots and tops removed. After the crop is harvested the spots where the diseased plants grew can be drenched with a 1–100 formaldehyde solution.

Lettuce plants 6 weeks old from seed may be set safely 8 days after the application of a 1–100 formaldehyde solution to the soil providing the soil is stirred 5 or 6 days after the treatment. But where only a few scattered spots are treated plants may be set as soon as the soil can be worked.

EFFECT OF FORMALDEHYDE ON SUBSEQUENT PLANT GROWTH

During the progress of this study it was manifest from a comparison of weights of lettuce plants of the treated and untreated plots that formaldehyde increased plant growth.

For example, 226 mature lettuce plants of a treated area in a commercial greenhouse weighed 164 pounds, while the same number of plants taken from an adjacent untreated plot weighed 132 pounds, which represents a gain of 23.6 per cent in favor of the plants grown on the treated soil. Clinton (*4*) also has noticed similar results on lettuce. Buddin (*3*) observed in his pot experiments with tomatoes that the crop was earlier and of greater bulk in the pots which had been treated with formaldehyde. That formaldehyde does increase plant growth must be accepted, but the exact reason for it has never been satisfactorily explained.

SUMMARY AND CONCLUSIONS

The maximum time required to kill small sclerotia with 1–50 formaldehyde solution in vitro was 15 minutes, and for large sclerotia 20 minutes. With 1–100 formaldehyde, small sclerotia, 15 minutes; large sclerotia, 30 minutes. With 1–200 formaldehyde, small sclerotia 75 minutes; large sclerotia, 90 minutes.

The maximum time required to kill both sizes of sclerotia is inversely proportional to the concentration of the formaldehyde solution.

In a 1–50 or a 1–100 formaldehyde solution in vitro, mycelium from pure cultures was killed in 5 to 10 minutes; in a 1–200 solution in 15 to 20 minutes.

Approximately the same length of time was required to kill sclerotia in both the 1–50 and the 1–100 solutions of formaldehyde; the same was true of mycelium.

Aspirating sclerotia for one-half hour in water and subsequent treatment in a formaldehyde solution in vitro failed to shorten the time in which they were killed.

Formaldehyde solutions are much more effective if the sclerotia are not in a desiccated condition. For this reason, the soil of commercial greenhouses should be maintained in a moist condition for 5 to 10 days immediately previous to the formaldehyde treatment.

The formaldehyde solutions greatly checked subsequent colony growth on nutrient agar of the sclerotia and the particles of mycelium which were treated in them.

In most cases the 1–100 formaldehyde solution killed sclerotia and mycelium 20 inches below the soil surface.

A 4-year test has demonstrated that a 1–100 formaldehyde solution is an effective soil fungicide when used to control *S. libertiana* in greenhouse soils. Sterilization is not always complete. In both experimental and commercial greenhouses the treated portions have not averaged over 2 to 4 per cent drop, whereas the unsterilized portions have averaged over 50 per cent drop.

In greenhouses that show only scattered cases of drop it is advisable to remove the diseased plants, mark their locations, and after harvest treat the infected area with a liberal application of a 1–100 formaldehyde solution. This will not delay work in the house, as the soil may be worked and planted the day following such treatment.

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PLATE I

A.—A lettuce plot in the experimental house affected with drop in 1917. The same year this plot was treated with a 1–100 formaldehyde solution. Nine crops of lettuce have since been grown in this bed without the loss of a plant.

B.—An untreated plot in the same bed as A and adjacent to it. Ninety-four per cent of the plants in this plot dropped.

A

B

DISTRIBUTION OF PENTOSANS IN THE CORN PLANT AT VARIOUS STAGES OF GROWTH [1]

By J. H. VER HULST, *Assistant*, W. H. PETERSON, *Associate Professor, Department of Agricultural Chemistry*, and E. B. FRED, *Professor, Department of Agricultural Bacteriology, Wisconsin Agricultural Experiment Station*

INTRODUCTORY DISCUSSION

Pentosans are known to be present in the cell walls of all green plants, in the bark and woody fiber of trees, in spices, fungi, mosses, various straws, tree and vegetable gums, fruits, and seeds. The wide occurrence of the pentosans in nature and the large percentage of furfural-yielding constituents in the cellular and skeletal structures of various plants has led many investigators to make a thorough study of these plant substances in the hope that some exact knowledge might be obtained concerning their origin and physiological function.

A study of the pentosan content of plants at various stages in their development discloses some significant facts. In most seeds the pentosans increase during the process of germination. This increase continues throughout the growth of the plant and is particularly marked in the fibrous portions such as the straw, the stem, or the leaf. The new tissue contains a smaller quantity of pentosans than the old; a young leaf may have only about two-thirds as large a quantity of pentosans as a dead and withered leaf. The formation of pentosans appears to parallel that of the cellulose and to be more particularly concerned with the structural requirements of the plant.

It is generally agreed that pentosans are not directly formed by assimilation of carbon dioxid. The increase in the actual quantity of pentosans in the germination stage indicates a conversion of hexose material into pentosans. This direct evidence for the conversion of hexosans into pentosans finds additional support in the discovery that xylans are in almost every instance found with glucosans, while arabans are most often found together with galactans. (*18, 14, 16, 6,*).[2] In many of the straws the xylose molecules are linked with the cellulose molecules in such a firm complex that they can only be separated by a strong acid hydrolysis.

Arabinose-galactose complexes (galacto-arabans) have been found in legumes, in arabinic acid, in cherry and peach gums, in gum tragacanth, and in coffee beans. De Chalmot (*2*) has pointed out the striking similarity between the stereoisomeric formulae of d-glucose and l-xylose, and between d-galactose and l-arabinose, and in view of this fact has proposed a theory to explain the conversion of hexosans into pentosans by the plant. According to this theory the pentoses are formed from the corresponding hexoses as a result of oxidation and decarboxylation of the terminal alcohol group. The proof for this theory has not been established, however, by experimental evidence.

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[2] Reference is made by number (italic) to "Literature cited," p. 662-663.

The question whether the pentosans may serve as reserve substances in the plants has been made a subject of study by numerous investigators. De Chalmot (*3*), Schöne and Tollens (*12*), and Götze and Pfeiffer (*7*) have concluded that the pentosans do not act as reserve substances. It has been shown that no decrease results in the pentosan content of plants during the different stages of development and hence the plant does not draw on the pentosans for the support of its life processes. Some exceptions appear to this general condition, as in the germination of the seeds of *Tropaeolum majus* (*2*), and in the decrease of pentosans in tree trunks (*17*) during the growing season. In a recent paper Tottingham, Roberts, and Lepkovsky (*19*) suggested that xylose-yielding substances may act as reserve carbohydrate in the metabolism of the apple tree. Miyake (*9*) determined the quantity of pentosans and methyl pentosans in the cotyledon and embryo of the germinated and ungerminated seeds of garden beans and soybeans and concluded that ordinarily the pentosans do not serve as reserve substances but that when the more easily utilizable material is used up the pentosans may function as reserve material. Schulze (*13*) and Ravenna and Cereser (*11*) are also of the opinion that in certain instances the pentosans may be used as reserve material.

Although the physiological function of the pentosans is still obscure and unsolved, it is doubtful whether their origin is the result merely of an accumulation of waste material. It is probable that they have some real importance in the formation of wood and in the formation of the skeletal structure of the various plants.

Associated with the pentosans are the methyl pentosans which have been found in mosses, algae, cereals, fungi, and in other plant material. Borghesani (*1*) made a study of the relationship between the amounts of pentosans and methyl pentosans in different species of soybeans and corn and found that this ratio was practically constant. However, little more than the wide occurrence and seemingly intimate relationship to the pentosans is known concerning the methyl pentosans.

A study of the pentosans and free pentoses in the corn plant at the various stages of growth seemed highly desirable, and these observations are described in this paper together with some data on the fermentation of the pentosans of green corn fodder by pure cultures of a number of bacteria.

EXPERIMENTS

Corn of the variety known as Golden Glow was planted in the greenhouse on January 15. The first plants were harvested on February 2 and were about 9 to 12 inches high. The plants were uprooted with the utmost care in order to obtain the entire root system and dry outer shell of the germinated kernel which is still attached to the plant at this stage of growth. These plants were not separated into tops and roots before determining the pentosan content. Some of the plants at the same time were harvested without the root system. Upon analysis it was found that the tops contained a considerably smaller percentage of pentosans than the whole plant. The plants of the succeeding stages were consequently divided into tops and roots.

The second series of plants was harvested on February 15. These plants were from 12 to 15 inches high and were just putting out their anchor or surface roots. They also still had the outer shell of the kernel attached to the root system.

The third series of plants was harvested on June 23 from a plot on one of the university farms. Because of the enormous increase in pentosans and dry matter in the plants which were harvested on March 9, it was felt that a series of plants should be selected which would represent a stage of growth intermediate between the series of February 15 and March 9, series 4. These plants were about 2 to 2½ feet high and were just putting out their anchor roots. On analysis, however, it was found that they were almost as far advanced as the series of March 9 and did not fit into the gap between the second and fourth series as well as could be desired.

On March 9, the fourth series of plants was harvested. These plants were about 3 feet high. All of the plants of the second, third and fourth series were divided into tops and roots.

The plants representing the next stage of growth were selected on March 26. They were about 5 to 6 feet high and were in the tassel stage. The plants were divided into roots, leaves, and stalks.

The sixth series of plants was harvested on April 12. These plants were in the silk stage and of about the same height as those of the preceding series.

On May 18 the plants of the seventh series were harvested. These plants were about 6 feet high and had produced ears which were in the milk stage.

The last series was selected on June 1. These plants were from 6.5 to 7.5 feet high, and the kernels were in the dent stage. The plants of the last three series were separated into roots, leaves, stalks, husks, cobs, and silk. In the last series the kernels were analysed separately from the cobs.

All the samples were thoroughly washed, dried, and weighed. A subsample of the material was ground to a fine powder and dried overnight at 105° C. in an electric oven. Duplicate samples of 1 gm. each were then taken, and a pentosan determination was made as described in the Kröber method (*8*). With these methods of sampling, grinding, and drying, duplicate determinations, checking to within 5 mgm. of phloroglucid were easily obtained. If a difference greater than 5 mgm. between duplicates was found, the determination was repeated. The results of this series of experiments are given in Table I.

TABLE I.—*Pentosan content of the different parts of the corn plant at various stages of growth*

[Calculated on the basis of 100 plants.]

Series No.	Age.	Stage of growth.	Part of plant.	Dry weight.	Pentosans.	Total pentosans.
	Days.			*Gm.*	*Per cent.*	*Gm.*
	0	.....	Kernels.....	29.6	7.4	2.2
1	18	9 to 12 inches.....	Whole plant.....	23.7	15.4	3.6
2	31	12 to 15 inches.....	Tops.....	16.7	11.6	1.9
			Roots.....	11.2	19.5	2.2
3	40	2 to 2.5 feet.....	Tops.....	495.7	15.1	75.2
			Roots.....	74.3	18.2	13.5
4	53	3 feet.....	Tops.....	559.4	17.3	97.2
			Roots.....	81.4	17.3	14.1
5	70	Tassel.....	Leaves.....	1,106.6	19.9	220.7
			Stalks.....	1,016.6	19.1	194.2
			Roots.....	350.6	19.8	69.5

TABLE I.—*Pentosan content of the different parts of the corn plant at various stages of growth*—Continued

[Calculated on the basis of 100 plants.]

Series No.	Age.	Stage of growth.	Part of plant.	Dry weight.	Pentosans.	Total pentosans.
	Days.			*Gm.*	*Per cent.*	*Gm.*
6	87	Silk forming	Leaves	1,374.8	17.6	242.2
			Stalks	1,213.4	16.3	198.7
			Husks	751.2	15.3	114.9
			Cobs	488.6	9.0	44.1
			Silk	126.8	9.3	11.8
			Roots	338.6	20.3	68.9
7	123	Milk	Leaves	1,958.2	18.1	356.2
			Stalks	2,405.2	16.5	398.8
			Husks	1,128.2	25.6	288.8
			Cobs	4,201.0	17.4	732.6
			Silk	126.7	14.5	18.4
			Roots	458.0	19.6	90.0
8	137	Dent	Leaves	1,493.0	19.4	290.3
			Stalks	1,950.5	19.5	380.7
			Husks	1,019.0	33.0	336.4
			Corn	3,554.2	6.4	227.4
			Cobs	1,212.5	31,8	386.1
			Silk	93.0	19.9	18.5
			Roots	615.2	21.8	134.4

It is apparent that there is an immediate increase in the absolute quantity of pentosans in the plant. Even at the first stage when the shoots are only about 10 inches high the increase in the quantity of pentosans in the plant over the quantity in the kernels is 66 per cent. This indicates the conversion of starch or other dry matter into pentosans. At the second stage, when the shoots are only a few inches taller, the increase has mounted to 80 per cent. At the third stage the increase is several thousand per cent and thereafter increases enormously at each stage of growth. It will be noted that at the first stage the dry weight as compared with that of the kernels is about 20 per cent less, as is to be expected. At the next stage the dry weight has again reached that of the corn kernels and thereafter increases, except for the last stage where the dry weight is slightly less than in the preceding stage. It will be noted that throughout the growth of the plant the pentosans increase proportionately with the dry matter, the weight of pentosans being approximately one-sixth that of the dry matter.

Another fact of interest is the variation in the percentage of total pentosans in the different portions of the plant at the various stages of growth. From 7.4 per cent in the corn kernel the percentage has increased to 11.6 in the tops, and 19.5 in the roots of the plants at the second stage of growth. From this stage to maturity the roots show only a slight increase. The tops have not as yet reached the maximum percentage and increase rapidly as the plant develops. As the different parts of the plant appear they contain a low percentage of pentosans, but there is a rapid increase as these parts develop. This is especially true of the cob, which shows an increase from 9 per cent to 32 per cent.

METHYL PENTOSANS

The various parts of the corn plant were analyzed for methyl pentosans. The method of Ellett and Tollens (5) was used in which the precipitate of phloroglucid is extracted with 95 per cent alcohol at 60° C. until extraction is complete. Results identical with those reported in an earlier paper were obtained. In three determinations of total pentosans the decrease in the weight of the phloroglucid (about 0.200 gm.) varied from 0.0006 to 0.006 gm., the average being 0.0037 gm. These determinations were made on 1 gm. of dried material and therefore indicate the presence of only about 0.37 per cent of methyl pentosans in the tissue of the corn plant.

Because of the general distribution of methyl pentosans in plant tissue which has been reported by other investigations it was deemed desirable to check our procedure by a determination on rhamnose.

TABLE II.—*Conversion of rhamnose into methyl phloroglucid under various conditions, and solubility of the phlaroglucid*

Sample.	Weight.	Yield of phloroglucid according to Kröbers tables.	Phloroglucid obtained.		Phloroglucid extracted by alcohol.
	Gm.	*Gm.*	*Gm.*	*Per cent.*	*Gm.*
Rhamnose	0.1	0.0587	0.0425	72.4	0.0425
Rhamnose	.2	.1364	.1041	76.3	.1041
Arabinose	.1	.0860	.0802	93.3	.0050
Rhamnose	.1	.0587			
Arabinose	.2	.1770			
Total		.2357	.2184	92.6	.0358
Plant material	1.0	.1672			
Rhamnose	.1	.0587			
Arabinose	.1	.0860			
Total		.3119	.2958	94.8	.0386
Plant material	1.0	.1672			
Rhamnose	.1	.0587			
Total		.2259	.2084	92.2	.0482

TABLE III.—*Destruction of the pentosans of green corn stover by pure cultures of various organisms*

Organism.	Source.	Pentose.	Pentoses destroyed.	
		Gm.	*Gm.*	*Per cent.*
Control		0.2146		
		.2150		
41–11	Soil	.1933	0.0215	10.0
		.2005	.0143	6.6
29	Sauerkraut	.2024	.0124	5.7
		.2046	.0102	4.7
124–2	do	.2030	.0118	5.4
		.2095	.0053	2.4
102	do	.2058	.0090	4.1
		.2111	.0037	1.7
31	Silage	.2101	.0047	2.1
		.2075	.0073	3.3
C–26	Green corn	.1878	.0270	12.5
		.1904	.0244	11.5
Bacillus flavigena	Fermenting cellulose	.1961	.0187	8.7
		.1873	.0275	12.8
3	do	.1904	.0244	11.5
Streptococcus lactis	Milk	.2159	.....	.....
		.2037	.0111	5.1
Bacillus coli communis	do	.1917	.0231	10.7
		.1887	.0261	12.1

A series of experiments was carried out with varying amounts of rhamnose and arabinose alone and together with 1 gm. of plant material. The sugars were dissolved in distilled water, and aliquots representing the required amount were added to the plant material.

The yield of methyl phloroglucid which was obtained did not equal that calculated from Kröber's table. However, all of it was extractable with hot alcohol. A somewhat larger weight was extracted when the rhamnose was added to the corn tissue, indicating the small amount of methylpentosans in the plant material. The increase, 6 mgm., is approximately equal to the phloroglucid extracted in the regular determination. While these trials showed considerable variation, it may be assumed that no great amount of methyl-phloroglucid was formed and remained unextracted in the regular determinations. The results are given in Table II.

DESTRUCTION OF PENTOSANS IN PURE CULTURE

About 50 gm. of corn stover were ground to a fine powder. One gram of the air-dried material was weighed into small flasks, 50 cc. of yeast water were added, and about 2 gm. of calcium carbonate to alternate flasks. The flasks were then sterilized and inoculated. The results are given in Table III.

It is at once apparent that some of the organisms destroyed the pentosans to an appreciable extent. The maximum destruction was accomplished with the cellulose fermenter, *Bacillus flavigena*. Nearly the same percentage of pentosans was destroyed by *B. coli communis* and No. C–26, a chromogenic pentose fermenter isolated from green corn tissue.

TABLE IV.—*Free pentoses in the corn plant at various stages of growth*

Series No.	Stage of growth.	Part of plant.	Pentoses.	
			Gm.	*Per cent.*
4	3 feet	Tops	0.067	0.73
5	Tassel	Leaves	.062	.69
		Stalks	.065	.69
6	Silk forming	Leaves	.047	.52
		Stalks	.154	1.66
		Husks	.078	.87
7	Milk	Leaves	.036	.39
		Stalks	.086	.92
		Husks	.033	.35
		Cobs	.024	.25
8	Dent	Leaves	.044	.47
		Stalks	.072	.76
		Husks	.024	.25
		Corn	.017	.18
		Cobs	.025	.26

In an earlier paper (*10*) pentoses to the extent of 0.6 per cent were reported in the stalk and cob of every green corn which was analyzed according to the method of Davis and Sawyer (*4*). A decrease of 6 mgm. could therefore be accounted for by a destruction of the free pentoses of the corn stover. In addition 5 mgm. must be deducted for the difference in the duplicate weights of phloroglucid. A loss of 11 to 12 mgm., or 5 per cent, of pentosans must therefore be attributed to these two factors. A destruction of more than 5 per cent, on the other hand, may safely be attributed to the destruction of the pentosans themselves.

FREE PENTOSES

Ten grams of the material were placed in an Erlenmeyer flask, together with approximately 5 gm. of calcium carbonate to neutralize the acidity and 100 to 150 cc. of 85 per cent alcohol. The samples were placed in a boiling water bath and refluxed. Two extractions of three to four hours were made. The insoluble material was filtered away and the alcohol evaporated. The residue was taken up with water, filtered, and finally distilled as usual with 12 per cent hydrochloric acid. The results are given in Table IV.

No extractions were made upon the plant material of the early stages because of the lack of material. It will be noted that the percentage of free pentoses is approximately the same in the leaves and stalks of series 4 and 6. The outstanding feature of Table IV is the high percentage of free pentoses in the stalks of the plants of the sixth series. Shaw and Wright (*16*) have published a table in which the amount of reducing sugar in the corn plant at the different stages of growth is given. At the stage at which the kernels are forming, which corresponds to series 6 in Table IV, they find the largest amount of reducing sugar, namely, 20.3 per cent. Coincidently, the highest percentage of free pentoses was found in the stalks at this stage of growth.

Further evidence that free pentoses are contained in corn tissue was obtained by fermenting the alcohol-extracted sugars with yeast. The extracted sugars were made up with suitable nutrients and inoculated with a pure culture of yeast. Vigorous evolution of carbon dioxid ensued for a few days, but soon ceased. After 15 days the fermented liquid was analyzed for reducing sugars. From 500 gm. of corn tissue 3.54 gm. of sugar calculated as xylose remained unfermented by the yeast.

SUMMARY

The pentosan content of the corn plant varies in different parts of the plant and at different stages of growth. From a percentage of 7.4 in the kernel, it increases throughout the stages of germination, growth, and reproduction until a percentage of 31.8 is reached in the cob at maturity. The several parts show minor fluctuations, but in general there is a steady increase both in the actual weight of pentosans and in the percentage of pentosans.

During the first 30 days after planting the weight of pentosans increased from 2.2 gm. to 4.1 gm. per 100 plants, although there was no increase of dry matter. Pentosans are evidently formed from starch or other dry matter. After 30 days there is a very rapid increase in both the pentosans and dry matter.

Only a trace (0.37 per cent) of methyl pentosans was found. Additions of rhamnose to the corn tissue were made, and the total pentosans were determined. Although a quantitative recovery was not obtained, the weight of phloroglucid soluble in hot alcohol was approximately equal to that obtained from both rhamnose and corn tissue separately.

Free pentoses were found to be present in the corn plant throughout its period of growth. They varied from 0.6 to 1.7 per cent of the dry matter. The maximum percentage was found in the stalks at the time when the kernels are forming. This is also the time at which the total sugars reach their maximum.

A destruction of the pentosans of green corn stover was obtained by pure cultures of a number of organisms. The maximum destruction was 12.8 per cent by a cellulose fermenter *Bacillus flavigena*. A chromogenic pentose fermenter occurring on green corn tissue also produced a considerable destruction of pentosans.

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STIMULATING THE GROWTH OF AZOTOBACTER BY AERATION [1]

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INTRODUCTION

Numerous theories and methods have been advanced for increasing the growth of the Azotobacter cell. Much attention has been directed toward the improvement of a medium, the dominating thought being that the common media used for azofication purposes are deficient in those elements which are necessary for the prompt development of the Azotobacter organism.

Söhngen (*4*)[2] states that in Beijerinck's medium only nitrogen and oxygen are lacking for the luxuriant growth of Azotobacter. A vigorous growth of these organisms could be induced by the addition of his (Söhngen's) colloids to this medium, the colloids causing a direct contact between the Azotobacter, oxygen, and nitrogen.

It is not doubted that the medium commonly employed in the cultivation of these organisms could be improved; there are few bacteriological media which could not be improved. On the other hand, it is not assumed that the medium exerts the greatest influence upon the tardy development of the Azotobacter.

The theory of Söhngen that the Beijerinck medium lacks only nitrogen and oxygen is here supported. Observations prompt one to believe that both these elements can be supplied by aerating the culture medium and that thereby a rapid and vigorous growth of Azotobacter can be promoted.

The influence of aeration upon nitrogen fixation by Azotobacter is not new. Its influence is taken advantage of in studying the azofication ability of the soil or pure cultures by inoculating media of shallow depth and large surface exposure. This Lipman (*1*) noticed in the cultivation of *Azotobacter vinelandi* Lipman, for the rate of nitrogen fixation increased with the increased surface exposed.

The relative importance of aeration upon the growth of Azotobacter can be surmised from some of its physiological activities. Its obligate aerobic characteristics as well as nitrogen requirements for the synthesis of cell protoplasm are significant. Inasmuch as nitrogen is lacking in the media, the utilization of atmospheric nitrogen is necessitated. The energy required for this assimilating process is supplied by the oxidation of a carbohydrate. The abundance of air therefore offers two highly essential elements, oxygen and nitrogen, both of which are required for the efficient metabolism of the cell.

Observations noted from the growth of Azotobacter cultures on dextrose-Ashby agar slants would not lead one to suspect the media as

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[2] Reference is made by number (italic) to "Literature cited," p. 677.

incapable of supporting rapid cell development. Such cultures have invariably shown a vigorous and heavy growth in 24 to 48 hours, a growth comparable to that exhibited by the common metatrophic forms growing on a nutrient medium. This rapid growth of Azotobacter on such agar slopes is attributed to maximum aeration.

The purpose of the present investigation was to develop a method for inducing a prompt growth of Azotobacter in liquid media. The importance of aeration in this respect is indicated by the following data.

METHOD OF AERATION

Hoffman (*3*) suggested the sand slope cultural method for supplying a maximum surface exposure for aeration. Allen (*7*) used the mechanical agitation method employed by Bonazzi (*6*) in his nitrification experiments and reported variable results with this method of aeration. He attributed the greatest influence to the chemical composition of the media.

The activity of organisms in many fermentation industries has been materially increased by aeration. Probably in most cases the interest has been focused upon the fermentation processes rather than on any direct interest in cell multiplication. However, within the last few years aeration has been employed commercially for the dirct purpose of increasing the growth and thereby the yield of microorganisms. The cultivation of baker's yeast and the production of yeast protein in Germany by the fermentation of factory wastes is dependent upon this principle.

Aeration was accomplished in the following experiments by bubbling air vigorously and continually through the culture medium. The air was passed through the culture by attaching the culture flask equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the culture was reduced to a minimum by filtering the air through a sterile cotton filter and two or three sterile flasks of water. The air was thus filtered through cotton and rinsed in water before entering the culture flasks.

Several flasks of culture media could be easily aerated at the same time. Usually a cotton filter and wash bottle were placed between each two culture flasks. Little trouble occurred from contamination. A good grade of rubber tubing and tight-fitting connections are necessary, however. Flasks containing from 200 cc. to 250 cc. of media were used. The amount of air passed through the cultures was not measured, but a vigorous bubbling of the liquid was maintained continuously.

AZOTOBACTER CULTURES

Cultures of Azotobacter were isolated from samples of soil received from various parts of Kansas, Colorado, California, Iowa, and Mississippi. Flasks containing dextrose or mannite Ashby media were inoculated with the soil samples. Upon the formation of the characteristic surface growth Ashby agar plates were streaked. Dextrose-Ashby-agar slants were inoculated from well-isolated colonies, and repeated streakings of the cultures upon Ashby agar plates were made. A large number of the cultures were streaked consecutively from 1 to 12 generations. The utmost care was exercised in attempting to obtain and maintain pure cultures. Much difficulty was experienced in satisfying one's self of the absolute purity of a culture. This is accounted for by several justifiable reasons.

The purity of the culture is dependent practically entirely upon morphological considerations. The evident complex life cycle of Azotobacter, with its varied and fluctuating forms, makes a morphological differentiation indefinite, especially since our present knowledge pertaining to this life cycle, and its relative importance to the physiological activities of the cell is limited.

In all, 16 cultures of Azotobacter were used in these experiments. No attempt has been made to classify them as to species. In general, cultures 232, 6A, 12B, 9B, 8B, 1B, 10B, and 19 were all typically Azotobacter organisms producing a brown to black pigment. Culture 3A, 213, 2B, 5B, 11B, 9A, 222, and 3B were likewise typical, but pigment production by them was absent or doubtful. Culture 6A and 9B were isolated from Mississippi soil, 10B from California soil, 5B from Colorado soil, and 3A from Iowa soil. The remaining cultures were isolated from soil obtained from various parts of Kansas.

The inoculum used for seeding media was prepared by adding a portion of the emulsion from a young dextrose-Ashby-agar culture to a flask of dextrose-Ashby broth. This was aerated for two to four days. The amount of this starter usually used for inoculating the experimental medium was 0.05 per cent to 1 per cent of the medium seeded. In all cases a morphological analysis of the starter was made as a test for purity before using. The temperature for incubation was 30° C.

MEDIUM

The medium used in the following experiments, unless otherwise noted, was:

	Grams.
Tap water	1,000.0
Potassium phosphate (K_2HPO_4)	.5
Magnesium sulphate ($MgSO_4$)	.2
Sodium chlorid (NaCl)	.2
Dextrose	10.0

This solution was neutralized, filtered if necessary, and the required amounts placed in flasks and sterilized in the autoclav at 20 pounds pressure for 30 minutes. A pinch of calcium carbonate ($CaCO_3$) was added to each flask before sterilization.

CHEMICAL ANALYSIS

Total nitrogen determinations and sugar analyses of the Azotobacter cultures were made at frequent intervals. In all cases the total nitrogen was determined by the usual standard methods. Unless otherwise noted, 50-cc. portions of the media were used in duplicate. The figures referred to in the tables denote the average of the duplicate analyses. Nitrogen is recorded in all cases unless stated as net gain in milligrams of nitrogen per 100 cc. of media. This, in other words, refers to the amount of nitrogen fixed per gram of dextrose, as 100 cc. of media contains this amount of sugar.

Sugar determinations were made according to the method proposed by Shaffer (5). The copper resulting from the reduced Fehlings was determined by colorimeter readings. Duplicate readings were made each time, and the average of these was recorded. As a general rule, 50 cc. of the media were used. The protein was precipitated and removed, the filtrate was diluted to 100 cc., and 20 cc. of this filtrate were used for reduction. The figures cited, unless so stated, refer to grams of dextrose per 100 cc. of medium.

EFFECT OF AERATION UPON NITROGEN FIXATION

Flasks containing 250 cc. of media were inoculated with six Azotobacter cultures. Altogether, 36 flasks of media were used. Six flasks were inoculated with each respective culture. Three of the flasks for each organism were aerated and three were not. Examinations were made on the second, fourth, and sixth day.

The invigorating action of aeration is conclusively shown by these data, given in Table I.

An average of the figures from the six cultures give a net gain of 5.02 mgm. nitrogen per 100 cc. of the aerated media in two days as compared with 0.48 mgm. nitrogen in the nonaerated cultures. In four days the comparison indicates an average gain of 8.5 mgm. nitrogen in the aerated cultures to 1.24 mgm. nitrogen in the nonaerated cultures. The average net gain of the aerated flasks for six days was 10.35 mgm. nitrogen to 3.25 mgm. nitrogen for the nonaerated cultures.

TABLE I.—*Effect of aeration upon nitrogen fixation*

Culture.	Milligrams of nitrogen fixed.					
	Aerated cultures.			Nonaerated cultures.		
	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
1 BM	5.3	8.9	11.5	0	2.0	6.6
3 BM	4.9	14.2	15.9	0.34	1.2	6.0
222 M	6.0	8.0	11.0	.68	2.1	6.0
10 BP	.....	7.1	8.1	.2	.2	.2
12 BE	4.3	5.6	8.4	.7	.7	.7
232 E	4.6	7.2	7.2	0	.....	.6
Average	5.02	8.5	10.35	.48	1.24	3.25

It is to be assumed that on account of insufficient surface exposure in the nonaerated cultures azofication would be tardy. Nevertheless the experiment demonstrates the stimulating effect of aeration and the possibility of cultivating the Azotobacter rapidly in large volumes of media.

To compare the relative efficiency of this method of aeration with shallow depth culture media the following experiment was performed. Eighteen 300-cc. flasks containing 50 cc. of dextrose media were inoculated with 0.5 per cent of inoculum of culture 19. These cultures were incubated and three flasks were removed for analysis each day for six days. Nitrogen determinations were made upon the contents of each of two flasks. The third flask was used for sugar analysis. Six 300-cc. flasks containing 250 cc. of the same media were inoculated with a similar proportion of culture and treated to forced aeration. One flask was removed each day for nitrogen and sugar determinations. This media contained no $CaCO_3$. The reaction of the medium was readjusted to a P_H of 7.0 to 7.4. The results are shown in Table II.

TABLE II.—*Effect of aeration upon nitrogen fixation in culture 19*

	1 day.			2 days.			3 days.		
	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.
Aerated	0.67	5.60	Alkalin..	0.06	10.20	Alkalin..	0	12.50	Alkalin.
Nonaerated	.848	0	...do.....	.60	4.50	...do.....	Trace.	11.26	Do.

	4 days.			5 days.			6 days.		
	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.
Aerated	0	12.90	Alkalin..	0	10.80	Alkalin..	0	10.80	Alkalin.
Nonaerated	0	12.06	...do....	0	10.80	...do.....	0	10.80	Do.

The effect of the forced aeration is noticed in the first three days of incubation by both the nitrogen fixed and the dextrose fermented. The amount of nitrogen finally fixed in the shallow culture flask was practically as great as in the aerated culture. This would naturally be expected, however, as such cultures offered a maximum surface exposure. However, nitrogen fixation and dextrose consumption was more prompt in the aerated cultures.

The daily reaction of the cultures was alkaline, with possibly one exception which was questionable. This was the nonaerated culture on the fourth day of incubation. The P_H for the cultures reported alkaline averaged between 7.0 and 7.4.

EFFECT OF AERATION UPON DEXTROSE FERMENTATION

The ability of Azotobacter to fix nitrogen is dependent upon the energy derived from carbohydrate fermentation. The effect of aeration upon dextrose fermentation and the corresponding nitrogen fixation is demonstrated by the data in Table III.

TABLE III.—*Effect of aeration upon dextrose fermentation and nitrogen fixation*

	Aerated cultures.											
Culture.	1 day.		2 days.		3 days.		4 days.		5 days.		6 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
12 BE	0.54	1.3	0.26	4.3	0.07	6.1	0.04	5.6	0.04		0.03	8.4
222 H	.61	4.6	.09	4.6	.04		.03	7.2	.03		.03	7.2
Average	.57	2.9	.17	4.4	.05	6.1	.035	6.4	.035		.03	7.8

TABLE III. *Effect of aeration upon dextrose fermentation and nitrogen fixation*—Con.

Culture.	Nonaerated cultures.											
	1 day.		2 days.		3 days.		4 days.		5 days.		6 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
12 BE.....	1	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7
232 H.....	1	0	.8	0	.8	.....	.7	.....	.7	.6	.7	.6
Average.....	1	.35	.8	.35	.8	.7	.75	.7	.75	.65	.75	.65

Flasks containing 250 cc. of media were inoculated and incubated under aerating and nonaerating conditions. An aerated and nonaerated culture flask was analyzed each day for six days. The daily increase of nitrogen fixed as well as the daily consumption of dextrose in the aerated and nonaerated cultures is shown.

The marked effect of aeration upon Azotobacter is again indicated by these figures. In the aerated cultures the consumption of dextrose is very rapid. An average of 95 per cent of the original amount of dextrose disappeared within three days. Such is not the case in the nonaerated cultures. The average of the two cultures indicates that only 20 per cent of the dextrose was consumed within six days in the absence of aeration.

The comparative effect of aeration and nonaeration upon azofication and dextrose consumption is more clearly illustrated in figure 1 by the curves made from these data.

The striking effect of aeration is thus plainly evident, both in the nitrogen fixed and in the dextrose consumed. A close relationship is evident between the dextrose fermentation and azofication.

RATE OF NITROGEN FIXATION AND DEXTROSE FERMENTATION

Observation was made upon the comparative rate of nitrogen fixation and dextrose consumption in aerated cultures.

Allen (*8*) contributes data from one experiment and concludes that the results show that the rate of carbohydrate (dextrose) consumption by Azotobacter does not proceed in a manner similar to the rate of increase in cell numbers. Some of his cultures were agitated by mechanical means. This agitation appeared to hasten dextrose consumption as compared with those cultures which were not agitated.

The results obtained from six cultures of Azotobacter are shown in Table IV. The organisms were inoculated into a bottle holding 1,500 cc. of 1 per cent dextrose medium. The cultures were aerated two, four, six, and eight days. Total nitrogen and sugar determinations were made at the end of these periods by removing aseptically 150 cc. of the culture from the bottle. Curves plotted from the average of these results in figure 2, demonstrate a correlation between the rate of nitrogen fixation and dextrose fermentation. In general the results are similar to those indicated in figure 1.

The data representing the general average of the figures in the table are recapitulated in Table V. It will be noticed that the rate of azofication per gram of dextrose for each period of two days, is practically the same, namely, 6.90, 9.37, 8.34, and 9.74 gm. of nitrogen.

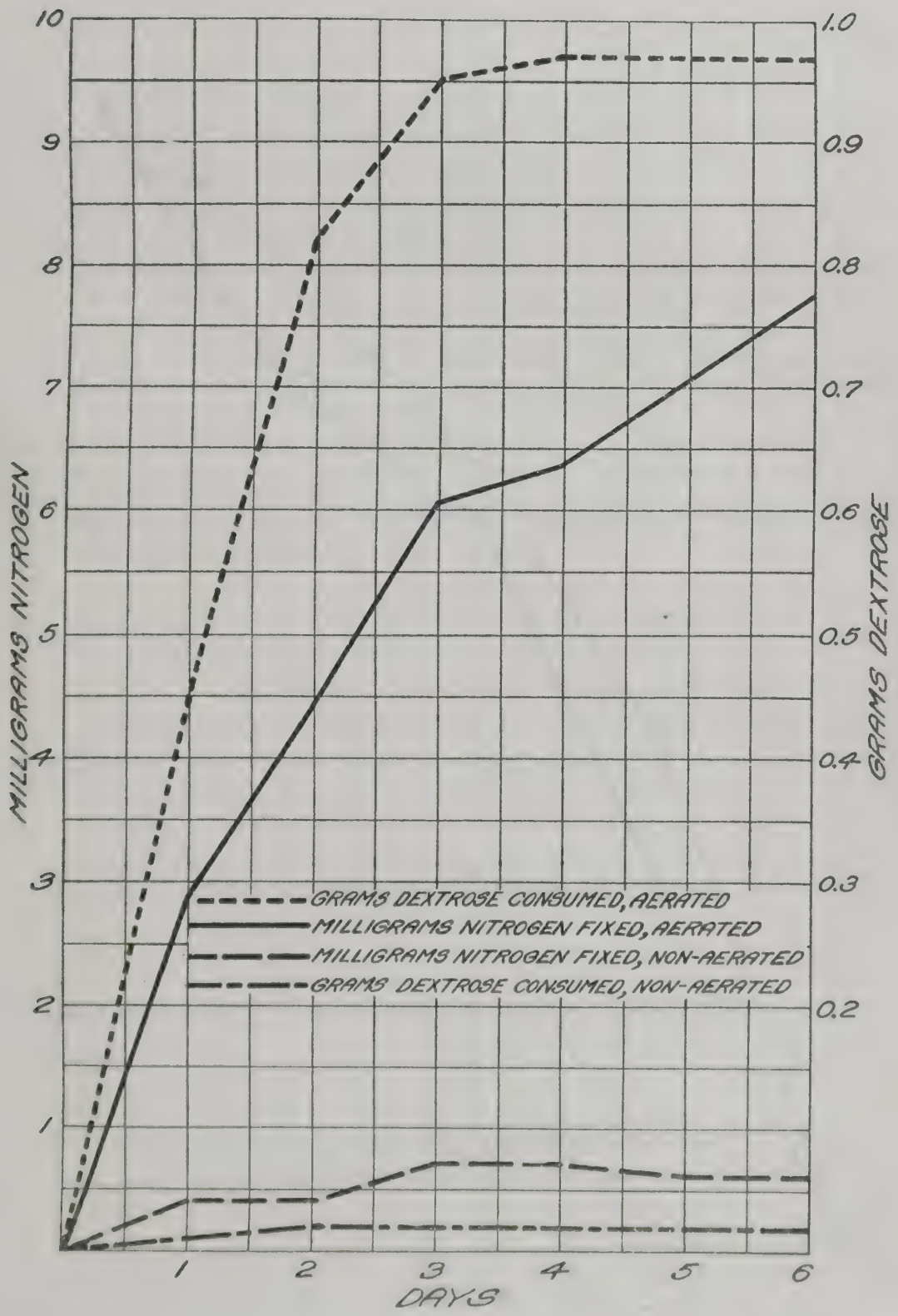

FIG. 1.—Effect of aeration on nitrogen fixation and dextrose fermentation.

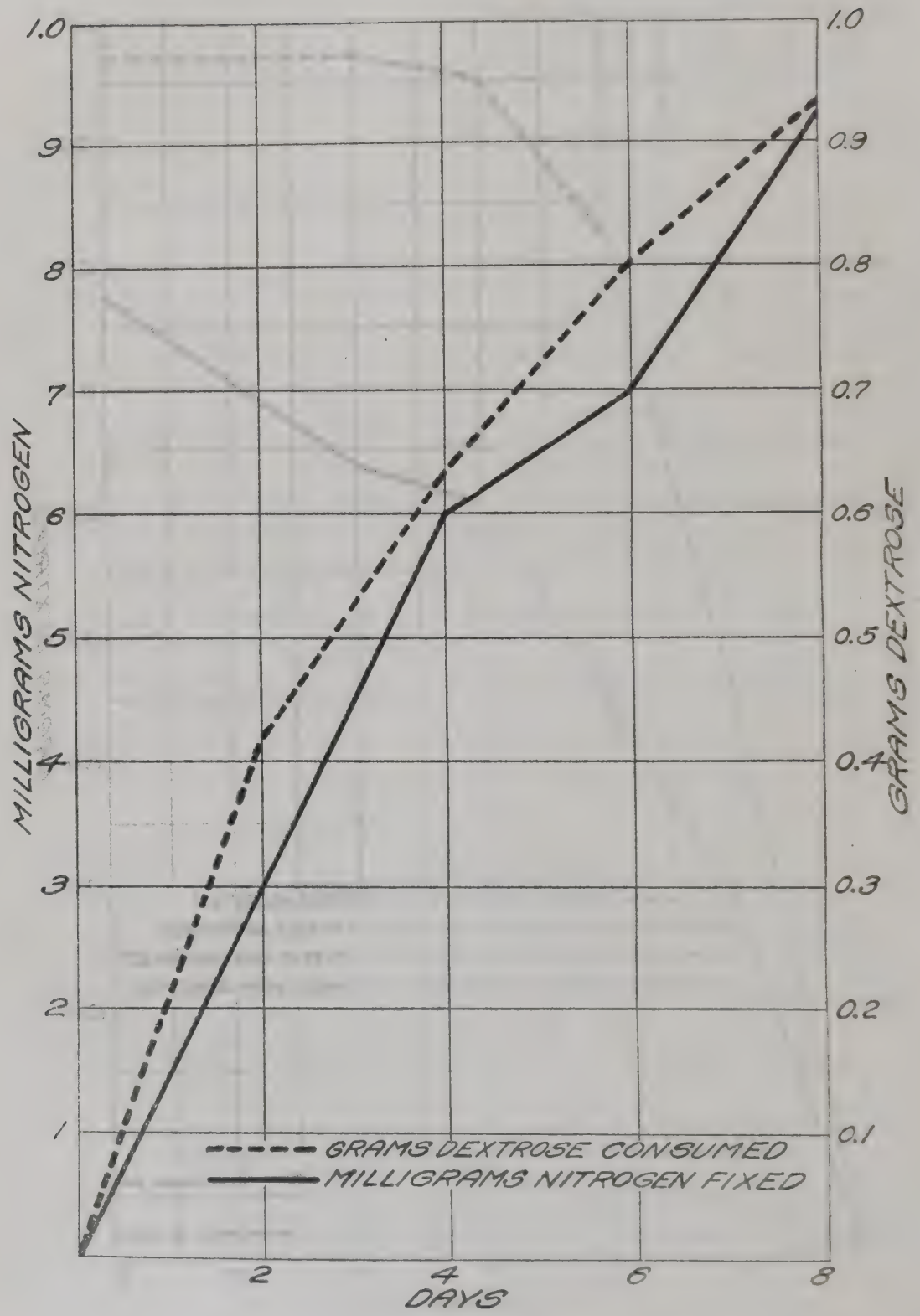

FIG. 2.—Rate of nitrogen fixed to dextrose consumed.

Examination also reveals that 42 per cent of the original amount of dextrose was consumed within two days, 22 per cent within four days, 17 per cent within six days, and 13 per cent within the eighth-day period, while 6 per cent was not fermented.

It also appears from these data that 31.6 per cent of the total nitrogen is fixed within two days, 33.8 per cent within the second period, 10.9 per cent within the third period, and 23.5 per cent within the fourth period. The amount of dextrose consumed in four days was 64 per cent of the sugar originally present, while the amount of nitrogen fixed within the same period was 65 per cent of the total nitrogen fixed. This periodical rate of nitrogen fixation and dextrose fermentation is noted in figure 3 by the curves plotted from these data.

Resummarizing the data in Table III as noted in Table VI shows that the rate of azofication per gram of dextrose per day is very uniform.

On the sixth day the amount of nitrogen recorded is greater than the amount actually fixed for this period as the nitrogen determinations were lost on the fifth day. The figures, therefore, in reality give the amount of nitrogen fixed between the fourth and sixth days.

During the first three days a fixation of 78 per cent of the total nitrogen occurred. The amount of dextrose fermented in the same period was 95 per cent of the original amount present.

The curves in figure 4, plotted from these data, exhibit curves similar to those noted in figure 3.

TABLE IV.—*Rate of nitrogen fixation and dextrose fermentation*

Culture.	2 days.		4 days.		6 days.		8 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
9 B	0.70	.....	0.49	6.6	0.28	6.7	0.10	7.0
2 B	.70	1.5	.49	8.6	.34	7.0	.13	7.4
9 A	.54	2.8	.24	8.0	.08	.....	0	11.6
5 B	.61	1.5	.49	5.6	.30	6.8	.2	9.8
11 B	.70	3.2	.49	4.9	6.21	5.3	0	10.0
8 B	.54	3.8	.49	4.9	.34	8.4	.3	.....
3 A	.50	2.9	.18	5.3	.04	7.7	.....	.....
213	.63	5.2	.32	5.6	.18	8.0	.....	.....
6 A	.38	5.3	.05	4.9	0	6.1	0	.....
Average	.58	2.9	.36	6.0	.19	7.0	.06	9.16

TABLE V.—*Rearrangement of data from Table IV*

	2 days.	4 days.	6 days.	8 days.
Total grams dextrose consumed	0.42	0.64	0.81	0.94
Total milligrams nitrogen fixed	2.90	6.00	7.00	9.16
Grams dextrose consumed each 2 days	.42	.22	.17	.13
Milligrams nitrogen fixed each 2 days	2.90	3.10	1.00	2.16
Rate of azofication per gram dextrose each 2 days	6.90	9.37	8.64	9.74

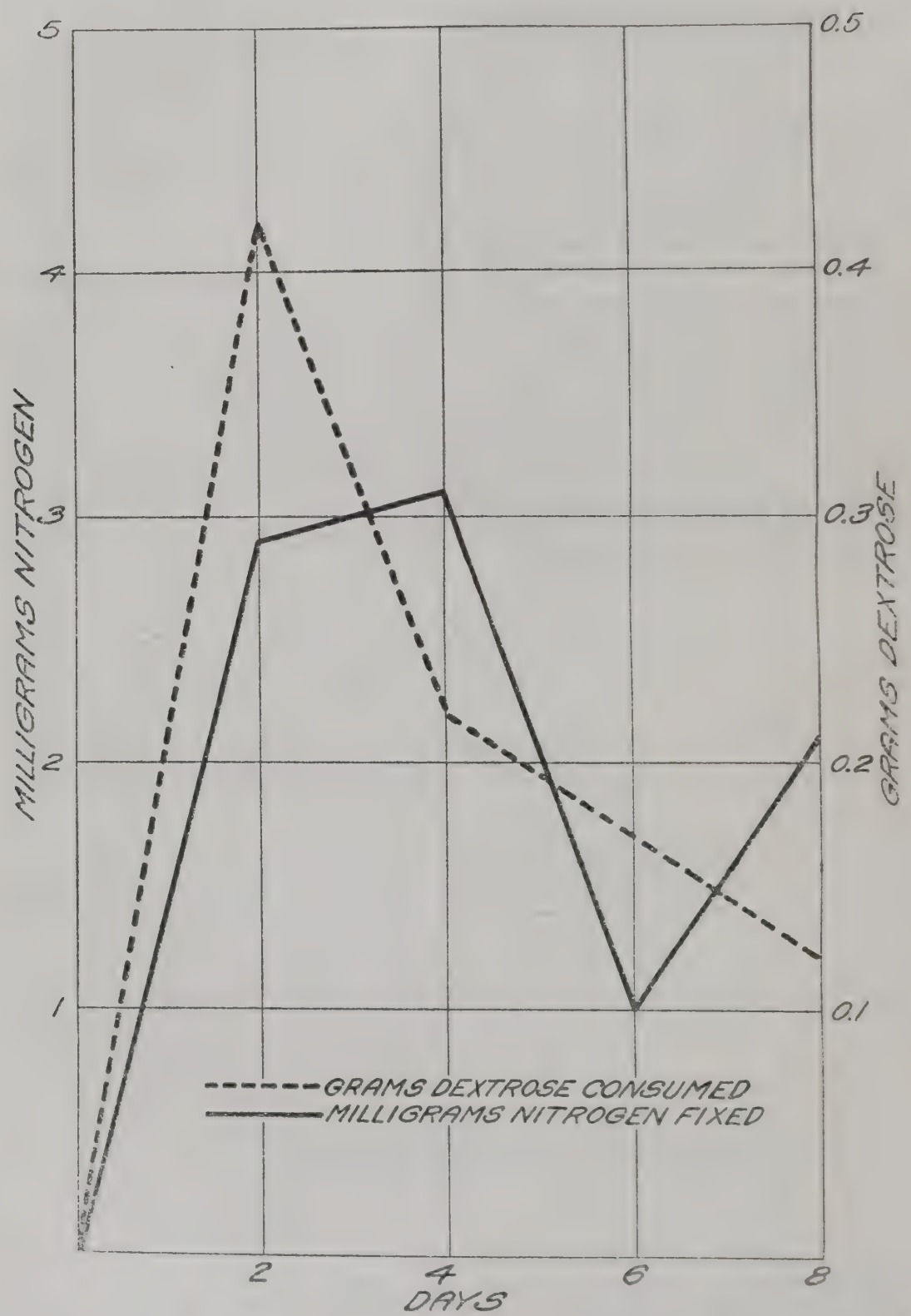

FIG. 3.—Daily amount of nitrogen fixed and dextrose consumed

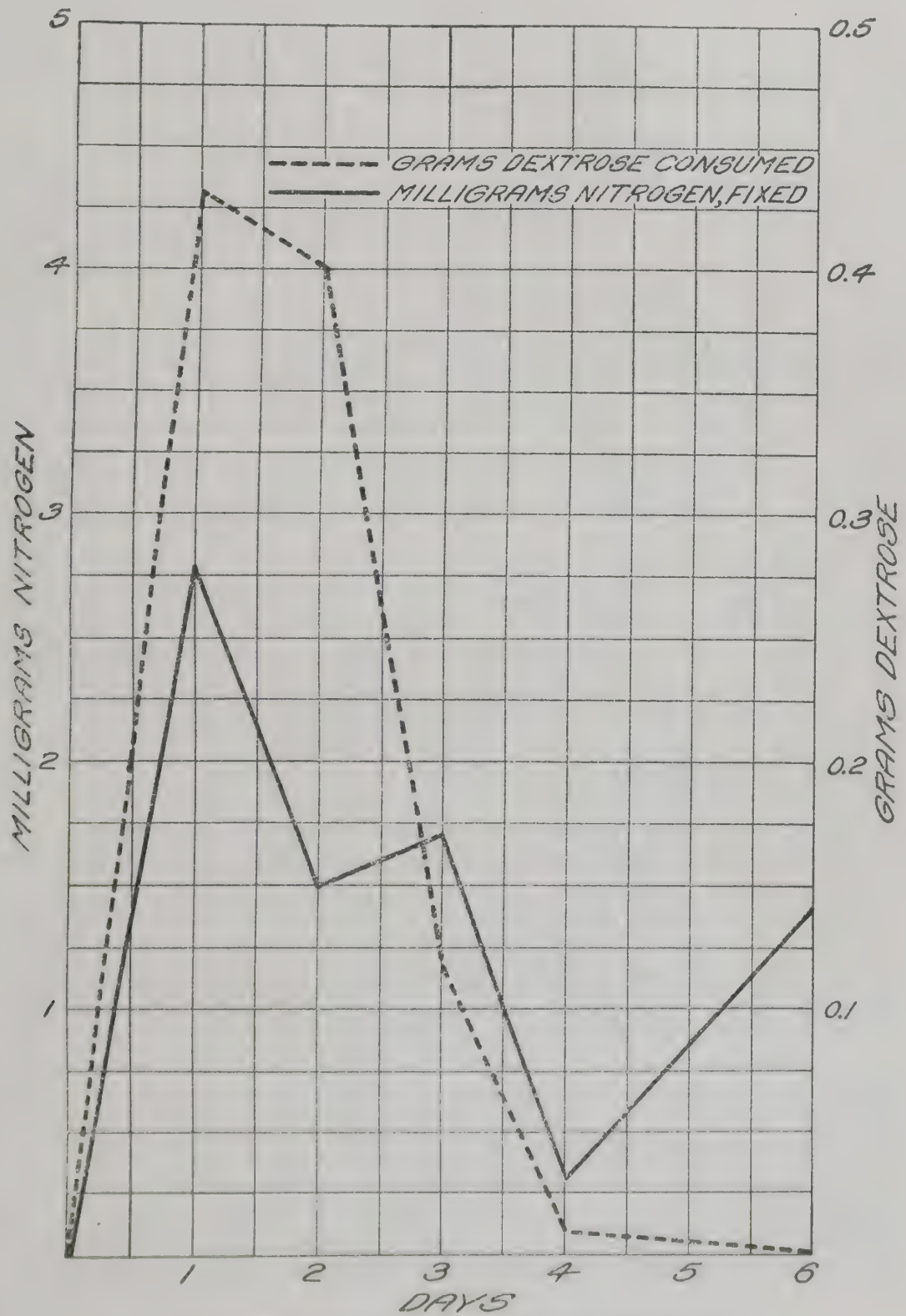

FIG. 4.—Daily amount of nitrogen fixed and dextrose consumed.

TABLE VI.—*Rearrangement of data from Table III*

	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.
Total grams dextrose consumed	0.43	0.83	0.95	0.965	0.965	0.97
Total milligrams nitrogen fixed	2.90	4.40	6.10	6.40	.....	7.80
Grams dextrose consumed per day	.43	.40	.12	.015	0	.005
Milligrams nitrogen fixed per day	2.90	1.50	1.70	.30	.....	1.40
Rate azofication per gram dextrose per day	6.51	5.30	6.42	6.56	.....	8.04

EFFECT OF $CaCO_3$

Calcium carbonate appears to be a universal constituent of the media used for the cultivation of Azotobacter. Lipman (*2*) asserts that calcium carbonate stimulates the growth of Azotobacter by either directly furnishing calcium or indirectly by making available more phosphorus, sulphur, and magnesium. Other investigators attribute its beneficial influence to the maintenance of a neutral reaction. Allen (*7*) in his studies on the composition of media for Azotobacter found $CaCO_3$ to be essential.

For further investigation with the cultivation of Azotobacter a substance fulfilling all the requirements of a good medium was desired—namely, one that provides the proper food constituents, proper reaction, and is clear and free from sediment. The presence of $CaCO_3$ interfered with the latter qualification. Preliminary experiments with a dextrose solution similar to the medium already mentioned, with and without $CaCO_3$, had furnished evidence supporting the doubtful value of $CaCO_3$ for the growth of Azotobacter in pure culture.

Flasks containing 250 cc. of media with and without $CaCO_3$ were inoculated with pure cultures of Azotobacter and aerated for six days. One flask of media was removed each day, and duplicate determinations of total nitrogen were made. The results are given in Table VII.

The averages of the analyses for two and four days indicate a slight increase in the amount of nitrogen fixed in the $CaCO_3$ medium. However on the sixth day the non-$CaCO_3$ medium shows a slight increase over the $CaCO_3$ solution. The slight increase of nitrogen fixed in one medium over the other is attributed to experimental error rather than to any marked effect of the media. These results are comparable with numerous others obtained in this laboratory. The conclusions are that with pure cultures undergoing a vigorous aeration the presence of $CaCO_3$ in the media is not essential for the prompt growth of the Azotobacter.

It should be stated that the medium used in these experiments and in all other work where $CaCO_3$ is not added was always corrected to a reaction near a P_H of 7.0 to 7.4. The reaction of such a medium inoculated with pure cultures of Azotobacter and aerated will vary but little.

TABLE VII.—*Effect of $CaCO_3$ upon nitrogen fixation*

Culture.	Milligrams nitrogen per gram of dextrose.					
	2 days.		4 days.		6 days.	
	No $CaCO_3$.	$CaCO_3$.	No $CaCO_3$.	$CaCO_3$.	No $CaCO_3$.	$CaCO_3$.
1 BM	4.3	5.3	7.0	8.9	9.1	11.5
3 BM	4.3	4.9	9.4	14.2	14.9	15.9
222 M	4.9	6.0	6.1	8.0	15.9	11.0
10 BP	1.7	.9	5.2	7.1	9.1	8.1
12 BE	.....	4.3	5.6	5.6	10.0	8.4
232 H	6.3	4.6	7.2	7.2	7.2	7.2
Average	4.3	5.0	6.75	8.5	11.03	10.35

SUMMARY

(1) A prompt and vigorous growth of Azotobacter can be induced in large quantities of liquid medium by sufficient aeration.

(2) Aeration stimulates rapid nitrogen fixation by Azotobacter.

(3) There exists a close correlation between the rate of dextrose fermentation and nitrogen fixation.

(4) The presence of calcium carbonate is not essential in a medium used for aerating pure cultures of Azotobacter.

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GRAY MOLD OF CASTOR BEAN[1]

By GEORGE H. GODFREY[2]
Pathologist, Cotton, Truck, and Forage Crop Disease Investigations

INTRODUCTION

The castor bean (*Ricinus communis* L.) has had a unique history as a commercial crop in America. From 1860 to 1900 it was a common crop, particularly in Oklahoma, Kansas, Missouri, and Illinois (*7, p. 295*).[3] Climate and soil conditions in some parts are highly suitable for its more general culture, as witnessed by the thousands of dooryard clusters of plants that may be seen throughout the Southern States. Furthermore, there has always been a sufficient home market for the product, the United States consuming annually something like a million bushels (*13*). Nevertheless production had gradually dropped off, and for years previous to the World War, the crop was grown only sporadically in limited areas in the South. The one factor which has prevented the crop from being a success in this country is an economic one, namely, inability to compete with foreign producing sections. The bulk of castor beans used in this country have for years come from India, where producing costs are low.

In one year, and for one year only, this state of affairs was completely changed. In 1918 large areas were devoted to the crop all the way from South Carolina to California, along the southern border. Something like 70,000 acres were planted altogether. The reason was, obviously, the great war, and the purpose, to provide the necessary lubricant, castor oil, for the rotary engines used in a part of the immense new fleet of aeroplanes. The usual source of supply, India, was virtually cut off by the war-emergency need of practically all shipping for other purposes. Consequently it was necessary that the supply be produced in this country. The first need was, of course, for an adequate amount of seed. Sufficient seed did not exist in America. This problem was met eventually through the activities of the War and State Departments, by having a shipload of castor beans from Bombay, India, diverted from England, where it had been destined to be made immediately into oil. The seed was distributed by an organization perfected by the War Department to farmers in the Southern States who agreed to grow them for a guaranteed price per bushel.

Early in the year before this unusual crop was planted a thorough search in the literature was made to determine to what diseases, if any,

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[2] The writer wishes to express his thanks to Mr. H. R. Fulton and Mr. J. R. Winston, of the Office of Fruit-Disease Investigations, for the use of the laboratory facilities at Orlando, Fla., where most of the investigations recorded in this paper were carried on. During two months, January and June, 1919, work on the problem was done at Cornell University, Ithaca, N. Y. The writer wishes to make special acknowledgment to Prof. H. H. Whetzel for the use of his own personal laboratory and greenhouse facilities, and for his invaluable suggestions and help throughout the course of the work.

[3] Reference is made by number (italic) to "Literature cited," p. 714-715.

the crop was subject. Only one was reported as occurring in America, a seedling blight in Mississippi in 1892 (*18*). Apparently but little work had been done on the disease at the time, and as the crop was not continued in later years no further investigations were made. The common dooryard castor bean of the South, "Palma Christi" as it is widely called, has always been so free from disease that it was commonly believed to be immune. In fact, there was a widespread superstition that it had a beneficial effect on other crops and even on chickens, around whose yards it is often planted. It was believed that the castor bean kept both kitchen garden and chickens from attack by insect pests.

Outside of America the most serious disease reported was a Phytophthora seedling disease and leaf blight in India, described by Dastur in 1913 (*6*). A few other minor diseases including a damping-off of seedlings, a rust, and a leaf spot, also occur in India. The diseases on the whole were not abundant and not serious. In this connection Dastur says in his paper (*6*, p. 178):

> Though the castor-oil plant is so widely distributed, still no important fungus pest, except the rust, has been recorded as attacking this crop.

Thus, in so far as plant diseases were concerned, the outlook was good for a successful season. Early in the summer, however, a number of pests appeared and required the attention of both entomologists and plant pathologists. The first alarm was caused by an outbreak of the semitropical army worm *Xylomyges eridania* Cr.[4] It practically stripped large portions of some fields of all green parts, resulting in the death of many of the plants. This was followed by the bacterial wilt, an account of which has been published by Smith and Godfrey (*14*). This, though widespread in its distribution, did not cause a very high percentage of loss. A few other insects made their appearance, particularly the red spider (*Tetranychus quinquenychus* McGregor) and the pink corn worm (*Pyroderces rileyi* Wals.)[4] In addition, two leaf spots made their appearance, one caused by *Cercospora ricinelli* Sacc. and Berl. (*4*) and the other by a bacterium. Neither was very serious. Finally, in July a gray mold appeared upon the inflorescence as the most serious menace of all. Members of the State Plant Board of Florida first reported its occurrence in different parts of the State to their headquarters at Gainesville. Entomologists who were combating the semitropical army worm noticed the increased seriousness of the gray mold as the rainy season advanced. The disease became more widespread and definitely more destructive. Specimens were sent to Washington to the Office of Pathological Collections, and the fungus was found to be a new species of Botrytis. Prof. H. E. Stevens, of the Florida Experiment Station, first published a report of the disease (*16*, *17*). A brief mention of it was also made in a Cuban publication (*4*). Later the writer, who was assigned to the investigation of the problem by the Bureau of Plant Industry, published a description of the causal organism (*10*).

Inasmuch as this castor bean disease was a new one in America, and as its development was gradual during the season, from a few sporadic primary infections to widespread destructiveness, the writer has attempted to treat the different phases of his investigations in so far as practicable in chronologic order, in order to better record its interesting history.

[4] KISLIUK, MAX, JR. INSECTS AFFECTING THE CASTOR OIL PLANT. (Manuscript in Bur. Ent., U. S. Dept. Agr.)

RANGE OF THE DISEASE

A preliminary survey was made early in August, 1918. The disease was found to be especially serious in Florida, which State was more extensively planted to the crop than any other. At Gainesville it was well scattered over a large field. At Orlando it had just made its appearance on a small scale. At Tampa, Seffner, and Fort Meade, it had already destroyed a considerable percentage of the blossoms and young pods and was rapidly spreading. The most serious outbreak of the trouble was at De Land, on the east coast. Here on August 10, approximately 50 per cent of the inflorescences were affected, and continuous moist weather gave promise of still further development of the disease. Farther south along the east coast, at Vero, West Palm Beach, Miami, and Davie, the disease had not yet made its appearance. In South Carolina, Alabama, and Georgia it was not to be found. In Louisiana, the first large field examined disclosed none of the fungus. In another field it was found in

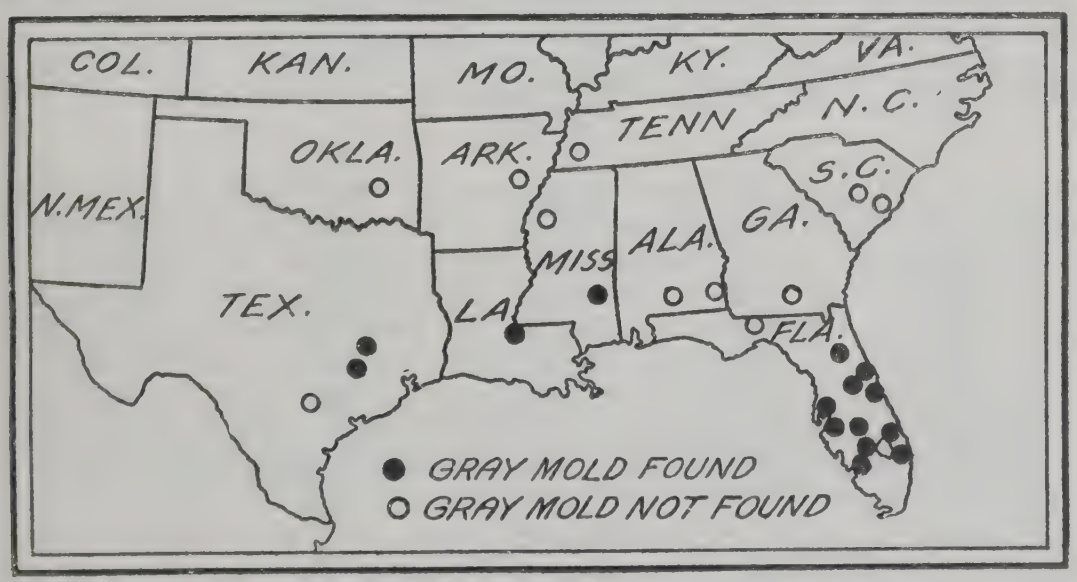

FIG. 1.—Map of the southern portion of the United States, showing the distribution of the gray mold of the castor bean.

abundance. The significance of this diversity became evident when it was learned that the first field was planted to seed grown in America the year before. The second field was planted to the usual Bombay seed. This feature will be emphasized later (p. 701) in connection with the origin of the pathogene. In southern Mississippi the disease was found in one field, in a locality that had been subject to recent heavy precipitation. It was reported by other observers who had become familiar with it to be absent from Texas, Arkansas, Tennessee, and northern Mississippi.

In September and October further surveys were made, to follow up the previous observations and to note any further spread. It was found to have become definitely established in fields at Kissimmee, Lucerne Park, Haines City, and at Moorehaven, on Lake Okeechobee, where a number of fields were seriously affected. In October a report of its occurrence in eastern Texas, following a protracted wet period, was followed up; and it was found that the disease was causing from 10 to 25 per cent loss in fields near Houston and Alvin. At a point in southern Texas not visited by the writer personally the trouble was not present, according to reliable reports. Here the whole summer was known to

be very dry. The absence of the disease in northern Louisiana, Arkansas, Tennessee, and northern Mississippi was further verified at this time by a personal survey. A field at Montgomery, Ala., was also found to be free from the disease. Neither had it occurred since the first visit, in plantings near Valdosta, Ga., Dothan, Ala., or Florence or McBee, S. C.

By November 15 the mold had become exceedingly destructive in localities where it had been seen earlier. At De Land, Fla., where it had been causing perhaps 50 per cent loss in August, it was now affecting practically every inflorescence shortly after it came out of the sheath, making the loss a full 100 per cent. It had also appeared at points along the east coast, particularly near Miami, and it was even found at the plantation at Davie, on the border of the Everglades. Here also it was found to have spread to scattered plants of "Palma Christi."

Figure 1 is a map showing the location of castor bean plantings in the South where the disease did and did not occur as determined by personal field inspections by the writer and reports from others. The losses in those localities where the disease was present varied from 10 to 100 per cent.

INFLUENCE OF WEATHER CONDITIONS ON THE PROGRESS OF THE DISEASE

During the course of the various survey trips through castor-bean growing sections, the writer noted the definite effects of moisture and temperature on the development of the gray mold. Its presence and rapid spread in the various localities in Florida during July, August, and September were definitely traceable to the warm moist weather of those sections, where the wet season regularly occurs in the summer time. In certain localities in particular it was observed that there were showers every day, frequently even several times a day. Plate 2, A, shows typical cloud effects that result in the showery weather referred to. At night there almost invariably occurred a heavy dew. Figure 2 is a copy of a figure in a report of the Weather Bureau (*19*) showing the average monthly rainfall in different sections of Florida. This excessive moisture made an ideal condition for the development of such a fungus as this one, a gray mold.

Figure 3, showing the daily rainfall (*20*) in particular localities for July, August, September, and October presents graphically the striking contrast in rainfall between typical localities where the disease was serious and those in which it did not occur.

Particular attention is called to the correlation between the summer rainfall and the occurrence of castor-bean gray mold. At Bartow, Fla., protracted wet periods occurred throughout the summer, and the disease appeared early and was continuously destructive. At Miami, Fla., except for one period early in July, at which time the plants were not yet fully developed, the wet weather did not begin until September 9, after which there was hardly a day without rain until after the middle of October. There the disease did not begin to be destructive until late summer. Again, at Houston, Tex., continuous heavy rain did not begin till about October 7, and there too, the disease was not reported until late in the season. At Valdosta, Ga., and Florence, S. C., where the fungus did not develop at all, the rainfall that did occur was of short duration and always followed by long periods of dry weather.

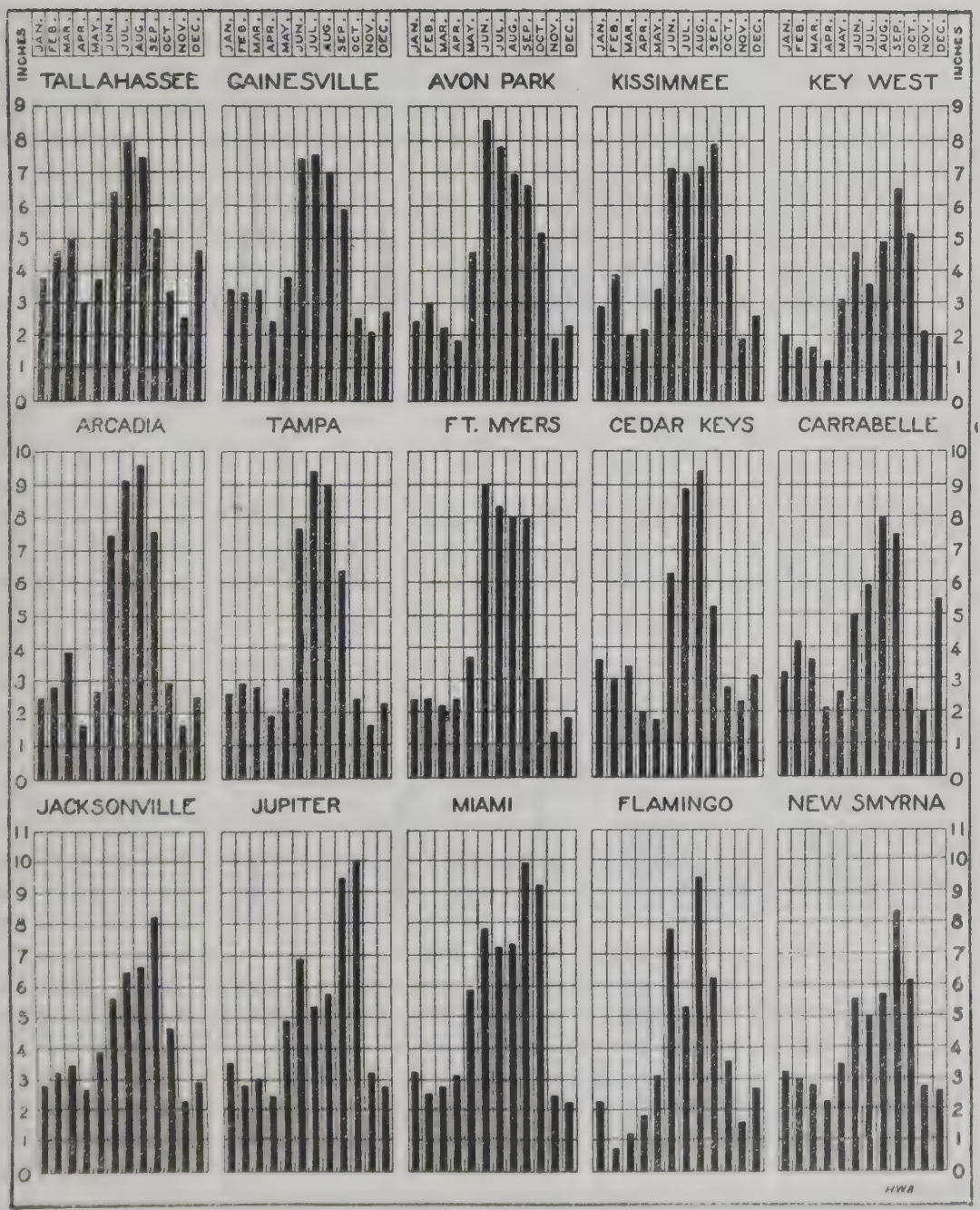

FIG. 2.—Diagrams showing the comparative monthly distribution of precipitation in different parts of Florida, the averages of several years' records.

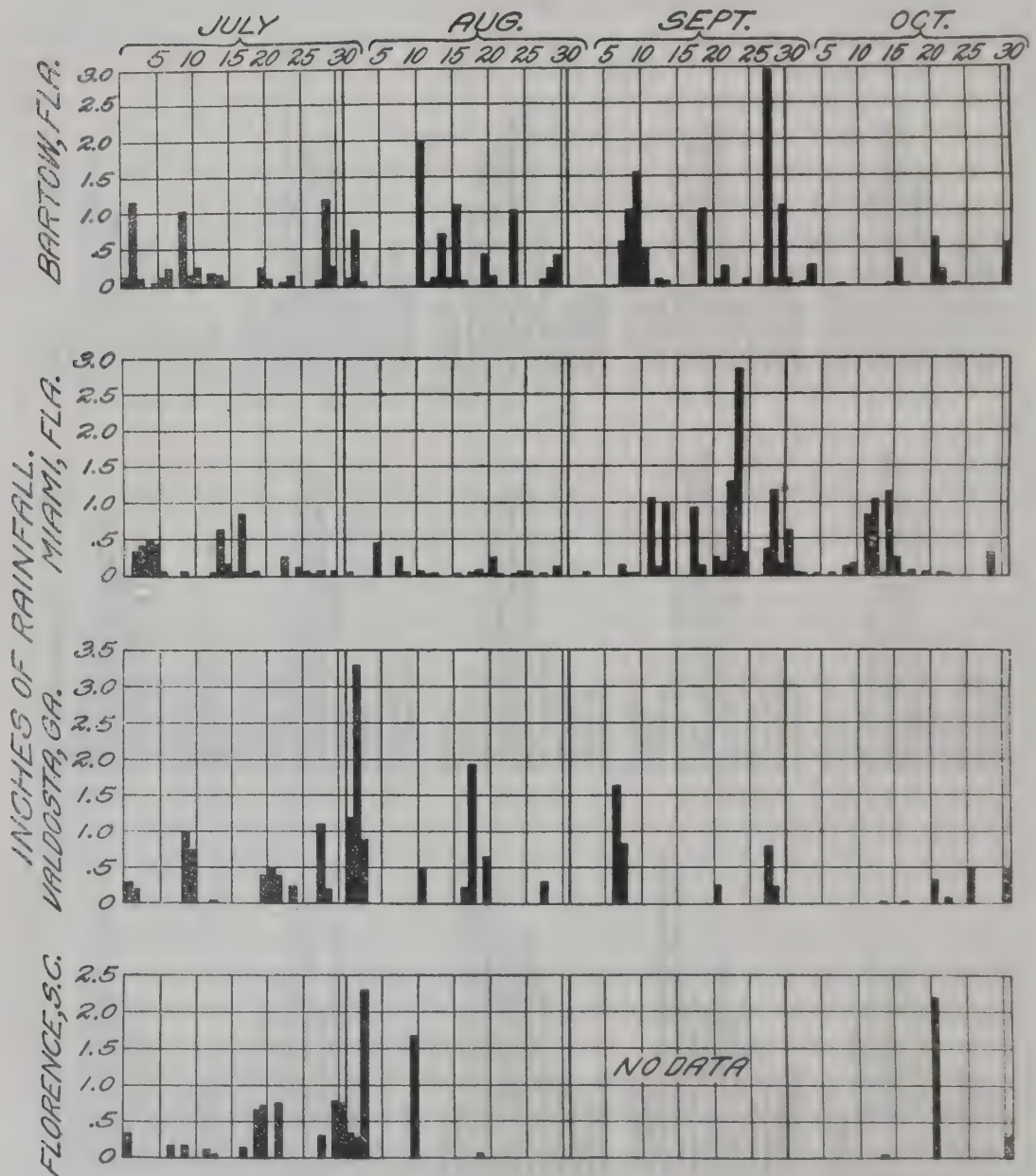

FIG. 3.—Daily precipitation records during the summer season, 1918, showing the difference in rainfall distribution between typical localities where the gray mold occurred (upper two) and where it did not occur (lower two).

The influence of temperature was not so definitely traced, though the indications are that it has its effect as well. As will be seen elsewhere in the paper, the optimum temperature for the development of the fungus in pure culture is approximately 25° C. (77° F.). Where temperature much below this prevailed in a locality otherwise favorable to infection it might be assumed that its failure to appear was due to too low temperature. Such a condition existed at Little Rock, Ark. After October 17, the moisture was favorable but the maximum temperature was above 72° F. on only two days, October 19 and 20, and it was 70° F. or lower practically all the rest of the month. The minimum fell below 60° F. several days. Likewise in Florida, with the cooler weather in the fall, the fungus definitely ceased to progress. It is likely that the fungus would not be nearly so active in a climate a few degrees cooler than Florida, even though moisture conditions were equally favorable.

DESCRIPTION OF THE DISEASE

Professor Stevens has given a very good description of the gray-mold disease in his Florida publications (*16,17*). A more complete description might well be given, however, especially of the earlier stages. Infection first becomes evident on leaves, panicles, or stems, in the form of small bluish spots, from which yellowish drops of liquid exude. Shortly after—in the course of only a few hours if weather conditions remain favorable—strands of hyphae may be seen extending from original infected spots along the surface of the host like a spider's web. These strands produce new points of infection. Development is rapid. The fungus infects constantly increasing areas on the host, meanwhile sending forth its fruiting hyphae. The inflorescence or (to use the common, though botanically incorrect terminology) "spike" becomes covered with this at first gray, then olive-drab mold. Plate 1 shows a typical young spike in an early stage of infection. Plate 3 shows three spikes with the mold farther advanced. At this stage the slightest jar will release a veritable cloud of conidia, which spread further destruction to the rest of the field. As an affected inflorescence becomes older, the mass becomes darker in color and more compact, and the stem droops and gradually loses its immature pods or blossoms. (Pl. 4, A, B.) Close examination of an infected spike will always disclose the presence of the mold itself, or some other characteristic feature of the disease to distinguish it from the natural drying of the male blossoms with which it is sometimes confused. The disease is sometimes still further complicated by the presence of the pink corn worm (*Pyroderces rileyi* Wals.), which is said to do injury at this stage.[5] The two conditions are often found together. In fact, specimens sent to Washington for examination were at first misleading because of the constant presence of this worm.

On the leaves, the disease is usually traceable to a small bit of an inflorescence that has fallen from above. From this central point the fungus spreads, producing a dead spot on the leaf, accompanied by the gray mold. Infection may also occur at the margin of a leaf, causing its death in characteristic Botrytis fashion. However, leaf infections are comparatively rare. Infections on the stalk and branches do not readily occur, the tissues here being more mature and consequently more

[5] KISLIUK, MAX, JR. OP. CIT.

resistant. Cases were seen, however, in which actual contact with a diseased inflorescence produced a distinct canker covered with the typical gray mold. In one case observed such a spot completely girdled the stem, killing the parts above, including several flowering branches and leaves. Plate 4 C, shows a canker on a stalk.

Unfortunately for the production of a crop of castor beans, the flowering parts of the plants are the most readily attacked by the fungus. A spike in any stage of development may become infected. If an abundance of the pathogene is present in the field and weather conditions are favorable, every spike will often become infected early in its development, even before the male blossoms have opened. More often, perhaps, infection takes place through the male blossoms. The anthers, water soaked as they often are after one of the summer rains or after a heavy dew, catch the spores of the fungus and permit them to germinate immediately and produce infection. Nearly mature spikes, containing only green pods, may become infected and fall over, maturing only a part or none at all of the beans. Usually a plant showed spikes in all stages, from buds just out of the sheath to nearly mature pods, all alike with evidences of recent infection.

The castor bean is characterized by its possession of extrafloral nectaries. These may occur at various places on the plant, often at the bases of leaf petioles, often on the leaf itself near the point of attachment of the petioles; but they are more numerous among the flowers. These nectaries exude nectar in large drops, which may be seen early in the morning before they have dried off. It was suspected that this nectar might favor infection by the fungus. Beneficial effect of nutrient material in the infection drop to infection by Botrytis has been mentioned by other writers (*3*) in connection with *Botrytis cinerea* Pers. Repeated examinations, however, failed to show that any large proportion of infections occurred in contact with these nectaries. The part played by the nectaries, however, is not small. They attract myriads of insects, principally honey bees, wasps, bumblebees, and ants which may be seen crawling through the spikes, infected and healthy alike, especially after a shower when conditions are ideal for infection. These insects undoubtedly play a large part in carrying infection. Whetzel (*22*) has noted such an insect relationship in connection with the spread of *Botrytis paeoniae* Oud.

The appearance of a badly infected field in the height of the growing season is indeed discouraging in so far as crop prospects are concerned. Plate 2 B shows a castor bean plant in which practically every inflorescence is affected. After the fruiting of the plant is thus arrested, new spurs shoot out with the brave intention of forming new blossoms. But almost invariably these new inflorescences which form throughout the summer sooner or later become infected.

As the growing season draws to a close with the advent of cooler weather in the fall, the plant gradually ceases to make new growth and with freezing weather becomes greatly weakened or dead. After the leaves are shed, the remains of the diseased spikes, with a suggestion of the still recognizable mold, may be seen (Pl. 5, A). Under the conditions of central Florida some life remained in many of the plants, and early in the spring with the gradually rising temperature, small, weak green spurs could be seen springing forth from various parts of the plants. Even earlier than this, however, evidences appeared of the overwintering of the fungus. On February 1, an inspection of the old plants standing in a

diseased field disclosed the first appearance of sclerotia under natural conditions. These were found upon the diseased spikes usually at their bases upon wood that still contained some sap. Plate 1 and Plate 5 A, show some typical examples of this condition. From this time on sclerotia could be found in increasingly greater abundance.

Before the middle of the month there were found sclerotia lower down on the stalk of the plant. It was soon obvious that spurs that had become infected during the first growing season were instrumental in carrying the infection into the cambium layer of the stalk. Here the fungus overwintered. With the advent of growing conditions, coupled with the decreased resistance of the host plant, it started again into active growth, which was semisaprophytic, in all probability, rather than purely parasitic. Sometimes a distinct canker could be seen, with a molded spur in the middle (Pl. 5, B, C). Sclerotia soon became evident, growing up through the cortex, sometimes evident on the surface only as a hump (Pl. 6, C), and again practically formed on the surface. Plate 6, A and B, are standing plants with an abundance of surface sclerotia. For the next two or three months stalk-borne sclerotia were to be found more and more. Many newly formed cankers with subsequent sclerotia were traceable directly to spring infections of new spurs that had started from buds on the stalks.

In early summer the coffee-bean weevil, *Araecerus fasciculatus* De G., listed among the corn-destroying weevils of the South by Cotton (5), was frequently seen on castor-bean plants of the previous season's growth, and the interesting observation was made that it was actually eating the sclerotia of *Sclerotinia ricini* Godf. Several of the weevils were caught and imprisoned and furnished with sclerotia and corn as food. They appeared actually to prefer the sclerotia to the corn. No significance is attached to their liking for this fungus as food, however, since they scarcely made an impression on the sclerotia that were available.

CAUSAL ORGANISM

ISOLATION

The organism was very easy to isolate. Any portion of a diseased inflorescence placed upon a poured plate of potato or corn meal agar at ordinary temperatures would develop the fungus rapidly, and as a rule it would outgrow all contaminating fungi or bacteria, and pure culture transfers could be readily made. In order to obtain definite strains of the fungus, however, the writer followed the precaution of obtaining single spore cultures in practically every case. This was done by lifting in a pair of forceps a small tuft of the fruiting fungus from the diseased material, holding it at the edge of the poured plate and giving a quick blow with the breath, thus blowing spores upon the surface of the plate. The method was suggested by Prof. H. H. Whetzel, who uses it largely in his work with Botrytis. Clear corn-meal agar was used by the writer. Inside of 24 hours well-isolated single spores, already germinating, were picked out and transferred to tubes. In case any particular plate was too thickly sown with spores, or an absolutely sure single spore isolation was in any other way doubtful, transfers were made to another plate of clear agar and the growth of the colony was followed to make sure of its purity.

In this way about 50 original isolations of the fungus were made. The sources and dates and particular information concerning them are listed in Table I.

TABLE I.—*Sources of cultures of Sclerotinia ricini*

Culture (B) No.	Date collected.	Isolation method.	Place collected.	Remarks.
	1918.			
1	Aug. 17	Tissue fragment	De Land, Fla.	Isolated in Washington by Ruth F. Allen.
2	Aug. 30	do	do	
3	Oct. 16	Single spore	Orlando, Fla.	Young inflorescence.
4	do	do	do	Do.
5	do	do	do	Large young inflorescence bud just out of sheath.
6	do	do	do	
7	do	do	do	An old infection.
8	Oct. 21	do	Haines City, Fla.	
9	do	do	Lucerne Park, Fla.	
10	do	do	Winter Haven, Fla.	
11	do	do	do	A half-mature inflorescence.
12	Oct. 23	do	Moore Haven, Fla.	
13	do	do	do	
14	do	do	do	
15	Oct. 29	do	Houston, Tex.	Sheldon district.
16	do	do	do	Do.
17	do	do	do	Do.
18	do	Sclerotium	do	Isolated Dec. 5 from sclerotium in above material.
19	Oct. 30	Single spore	Alvin, Tex.	
20	do	do	do	
21	Nov. 25	Tissue fragment	Orlando, Fla.	From male blossoms.
22	do	do	do	From inflorescence.
23	do	do	Tampa, Fla.	Do.
24	Nov. 27	Single spore	Sanford, Fla.	From "native" castor beans.
25	Dec. 9	do	Miami, Fla.	
26	do	do	do	
27	Dec. 10	do	do	From typical inflorescence.
28	do	do	do	From fallen pod.
29	do	do	Miami, Fla., Larkin (Fla.) district.	Do.
30	do	do	Allapattah, Fla.	
31	Dec. 15	do	do	
32	do	do	do	
33	do	do	Miami, Fla., Buena Vista (Fla.) district.	
34	do	do	Miami, Fla. (9 miles north).	
35	do	do	Davie, Fla.	From Bombay bean.
36	do	do	do	From "native" bean.
37	Oct. 30	Sclerotium	Alvin, Tex.	Isolated Dec. 23.
	1919.			
38	Feb. 7	Single spore	Orlando, Fla.	Winter fruiting of the fungus.
39	do	Sclerotium	do	Collected in the field.
40	Apr. 4	Tissue fragment	do	From lesions on spring-infected plants.

TABLE I.—*Sources of cultures of Sclerotinia ricini*—Continued

Culture No.	Date collected.	Isolation method.	Place collected.	Remarks.
	1919.			
41	Mar. 16	Not known.....	Santiago de las Vegas, Cuba.	Received from S. C. Bruner, Pathologist.
42	...do.....	.....do.....	.....do.....	Do.
43	May 1	Single ascospore....	Orlando, Fla.....	From apothecia on field sclerotia.
44	May 29	Tissue fragment....	.....do.....	Reisolation from plant inoculated with ascospore culture.
45	Dec. 15	.....do.....	Miami, Fla.....	From seed of lot 33.
	1918.			
46	Oct. 24	.....do.....	Plant City, Fla.....	Isolated May 27 from light-weight seed from hulling machine.

All these cultures were identical, showing conclusively the association of this one fungus with the castor-bean blight. Most of them, together with bits of the material from which they were isolated, were sent to Prof. Whetzel, at Cornell University, who verified their identity.

APPEARANCE IN CULTURE

Growth of the fungus on artificial media is very rapid. It is spreading and superficial at first, presenting a characteristically glistening appearance on the surface of the medium. Within 48 hours aerial hyphae begin to develop, in the form of erect single strands, the conidiophores. These develop very thickly and produce conidia often as early as the second day of growth. Soon the culture attains a light-gray color from the production of conidia, and as these become more abundant, it gradually becomes darker till it is drab or even dark-olive gray.[6] Figures A and B of Plate 10 are photographs showing the appearance of the fungus on corn meal agar three days after inoculation. About the fourth day sclerotia begin to form at the edges of the culture in a tube. These are anywhere from ½ mm. to 3 or 4 mm. in length, at first pale smoke gray[5] then gradually darker in color till they are black. At first flat and closely appressed to the surface, they gradually become wrinkled and distorted. On a highly nutrient medium, such as oatmeal agar, they are very abundant; on a less nutrient medium, such as one made with a small quantity of corn meal, less so. Appressoria, while present, are usually too small to be seen distinctly with the naked eye. Occasional cultures appeared in which they were massed together enough to produce the characteristic appearance of appressoria in a culture tube. (Pl. 11, A.) The writer's paper containing the description of the fungus (*10*) contains a drawing of a typical microappressorium.

The general appearance of the fungus in culture is that of a typical Botrytis with the usual abundance of gray mold and black sclerotia.

[6] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

MICROSCOPIC APPEARANCE

The development of a culture of this organism is comparatively rapid. The conidia germinate almost immediately on the surface of a poured plate by the development of a germ tube about half the diameter of the spore itself (Pl. 12, B). A cross wall is formed after the tube has grown in length to three or four times the diameter of the conidium, and profuse branching occurs immediately afterward, together with continued growth in all directions from the conidium. The mycelium of this fungus is easily distinguished from that of other fungi commonly found as contaminations in a plate by its large diameter and its general robust appearance. Its contents are distinctly granular and vacuoles are rare except in old cultures. Anastomosing of hyphae is frequent. It may occur between two hyphae which have arisen from the same spore or between two adjacent hyphae from different spores. Plate 13, I and J, are camera-lucida drawings showing anastomosis. The conidiophores may be distinguished in a young culture very early in their development. They arise perpendicularly from a cell of a horizontally growing vegetative hypha, becoming larger and olivaceous in color a short distance above the surface. Plate 12, D, is a drawing of the base of a conidiophore, showing its attachment to the vegetative hypha. Sometimes a single cluster of conidia is produced on the end of such a conidiophore. More frequently, however, the tip will divide and two equal branches will continue the growth. Often these will divide again, and a third or even a fourth division may occur, always dichotomous, the internodes becoming shorter by about half with each subsequent division. Finally the conidial head starts to form, this too by continued dichotomous divisions of the tip (Pl. 12, A). It was difficult at first for the writer to determine clearly the definite mode of attachment of the conidia to the ultimate branch tips of the conidiophore. Finally, by studying the development of very young cultures, this point was made clear. The conidia are borne on very fine attenuations of the ultimate tips of the conidiophore, these final cells being very turgid though thin-walled and fragile. No wall was discernible. Plate 12, C, depicts immature conidia attached to the conidiophore. Upon the maturity of the conidia, or upon the forcible detachment of nearly mature conidia, the ultimate cells of the conidiophore collapse and hang in a shapeless mass on the end of the next branch back, this tip becoming rounded out meanwhile, owing to the turgidity of the cell. It was impossible to see on a mature fruiting branch the exact connection of a mature conidium, owing to the density of the cluster of conidia.

Microconidia are formed in abundance, beginning after the cultures are about 10 days old. The writer never observed them in nature. Media favorable to heavy conidium production, such as corn meal or potato agar, sometimes do not readily produce microconidia. Upon media which do not produce conidia abundantly, such as oatmeal agar, cooked potato plug, and boiled rice, the microspores may often more readily be found. The writer has observed that they may often be found upon the thin piece of medium which has carried the transfer from one plate or tube to another. Microconidia are extremely small, from 2 to 3μ in diameter. The manner of production was readily observed, however, by examining the exposed surface of a poured plate, with 0.5-mm. water immersion objective. Considerable variation was noticeable in the type of conidiophore on which they were borne. Perhaps the

most frequent form was that pictured in Plate 12, F, a reproduction from a camera-lucida drawing. This is a special branch arising from the sterile hypha imbedded in the substratum. In another type the cluster of conidiophores arises directly on a cell of the sterile hypha (*10*, *Pl. XLI*, *e*). In still another form a long, slender branch bears upon its tip either a single conidiophore or a group of them, with the microconidia on their tips. Microconidia were observed to best advantage on germinating ascospores, as shown in Plate 12, G, and photomicrographically in Plate 10, C. From one or two to more than a dozen microconidia may develop in succession on the end of a single conidiophore, those that are mature dropping off and forming a distinct group. In general, the microconidia are very similar in all respects to those described by Brierly (*2*) for *Botrytis cinerea*. No germination was seen, however, though looked for diligently. No endoconidia were observed.

It was not deemed profitable to grow this organism on a long series of culture media, since its growth was almost equally vigorous on most media commonly used in the laboratory. There were a few specific differences evidenced that should be recorded. On corn-meal agar heavy production of the gray mold occurred, with comparatively little development of white mycelium and only occasional sclerotia. On potato agar, also, sclerotia were not abundant, but more mycelium was evident. Oatmeal agar at first produced a profusion of conidia, but later this appearance was hidden by more or less of the white mycelium and a great abundance of sclerotia. This was the medium most commonly employed for the development of sclerotia. Boiled rice and potato plugs had fewer conidia, but sclerotia were abundant. Beyerinck's agar produced a thin growth of conidia and few sclerotia. Sterilized sweet clover stems produced large sclerotia and few conidia. The interesting fact was noted that some media at times produced a heavy mat of conidiophores and conidia but no sclerotia at all. Transfers were made from such tubes to oatmeal agar to determine whether this was a character peculiar to the particular strain of the fungus. In every case a normal heavy development of sclerotia was produced on the new medium.

The cardinal temperatures for the growth of the gray mold on corn-meal agar are as follows: Minimum, 12° C.; optimum, 25° C.; maximum, 35° C.

DEVELOPMENT OF THE PERFECT STAGE

PRELIMINARY EXPERIMENTS

Before there had been any intimation of the existence of a perfect stage of this organism, attempts were made to induce sclerotia to fruit or otherwise function.

EXPERIMENT I.—On February 6, at Orlando, Fla., three Petri dishes of moist sand were sterilized and sclerotia from cultures of strains B 35 and B 37 placed thereon, and the whole placed in an incubator at 27° C. Observations were made for several days without noting any developments other than the production of gray mold from the medium at the edge, that had been carried over with the sclerotia. The plates became dry, and finally on March 16 sterile water was added and the plates were further treated as follows: No. 1, in the ice box for 24 hours, then under a glass moist chamber out of doors under fluctuating but on the whole fairly warm temperature conditions. No. 2, submitted to a −10° C. temperature in a freezing mixture for a few hours, the temperature gradually rising to +10° C., after which it was placed out of doors along with No. 1. No. 3, was placed directly under the out of door conditions.

Observations made frequently thereafter failed to show any signs of the development of a perfect stage.

EXPERIMENT II.—February 22. Sclerotia collected from castor-bean plants under natural conditions were sterilized for two minutes in 1 to 1,000 mercuric chlorid, washed in sterile water three changes, and placed partly on plates of agar, partly on plates of moist sterilized sand. The agar plates developed a typical culture of the fungus (B 39, isolation record, p. 688). On the sand, the mature sclerotia produced an abundance of normal conidiophores and conidia.

FIRST APPEARANCE OF APOTHECIA

The first appearance of the perfect stage of this organism was more or less unexpected; consequently it might be of interest to record its history in detail. On one of the cultures sent to Prof. Whetzel at Cornell University, No. B21, collected November 25, 1918, he noticed an apothecial stalk arising from one of the sclerotia. Prof. Whetzel immediately notified the writer at his temporary station, Orlando, Fla., and he found (Mar. 8) in his culture of the same strain (B 21) and also in one other strain (B 1), a similar condition. The cultures had remained on the shelf and had not been examined for some time. These cultures were then watched with jealous care at both places. In the culture at Cornell the stalk developed a little more, then shriveled and dried up. Under what were undoubtedly the more favorable conditions at Orlando, Fla., the culture continued to develop until the cup opened out and reached maturity. Thus there was secured a perfect specimen of the apothecial stage of this fungus which until then had appeared to be merely a new species of Botrytis.

Over anxiety to permit this specimen to reach full maturity resulted in its becoming slightly overmature, as was evidenced by the development of a mealy condition of the disk. Other apothecia were meanwhile rapidly developing, however, and it was clear that working material was to be had in sufficient abundance for immediate needs. Extraordinary precautions were taken with this culture tube to obtain absolutely authentic single ascospore cultures, for it was not known at this time whether or not further specimens could readily be obtained. From the first specimen to mature, mounts were made and drawings and measurements secured. The second was used to start cultures, but these were discarded in favor of the third, a more perfect specimen, from which more clearly reliable results were secured. This apothecium, taken in situ, is shown in Plate 11 A. For the sake of subsequent references it will be designated apothecium C. Its maturity was indicated on March 27 when a cloudiness different from that due to moisture was observed on the inside of the tube near the disk of the apothecium. A scraping was made from this deposit with a sterile platinum needle and examination showed it to consist of long fusoid spores, obviously ascospores that had been ejected from the mature apothecium.

In preparation for the actual work of securing single ascospore cultures, several Van Tieghem cells, slides, and thin cover glasses were sterilized in the hot air oven, within Petri dishes. In addition, tubes of sterile water and sterile vaseline were made ready. Then some of the cover glasses were covered within a small circular area on one side with a thin, smooth film of clear corn meal agar, applied directly from a tube of melted agar with a platinum loop. Apothecium C was then removed from the tube and placed in a sterile Petri dish.

Isolations were made from ascospores derived in three different ways, as follows: (a) Spores ejected directly from the apothecium to an agar-

coated cover slip, ejection being stimulated for the moment by a slight touch on the edge of the desk; (b) a segment cut from the side of the disk and crushed in a drop of sterile water, the resulting suspension of spores being then applied directly to the agar surface of a cover slip with a platinum loop; (c) spores scraped from the side of the tube to which they had been shot when the apothecium was first mature; these were applied as in (b).

In each case, the cover glass was then placed in position over a Van Tieghem cell which had previously been made ready by ringing above and below with sterile vaseline, sealing to a sterile slide, and pouring in a few drops of sterile water. A sterile moist chamber was thus secured which could be moved about as much as desired without fear of contaminating the contents. Several ascospores were observed, singly and in groups, and their germination and development were watched with the higher powers of the microscope. All were practically in one plane so that it was easy to mark with India ink the position of germinating single spores. After a few hours several such single ascospore strains were cut out and removed to a second agar-coated coverglass and cell, where their purity was verified and further development watched. The transfer was accomplished by lifting the coverglass between the thumb and forefinger of the left hand and cutting out the area marked, a millimeter or so square, with a No. 24 platinum-wire needle which had a very thin sharp, knife-like point. Later these single spore strains were transferred to tubes of agar.

Inasmuch as this method of isolating single spores is not used as widely as its practicability would justify, the writer wishes to call attention to it as a simple, quick, and reliable means of obtaining single spores apart from one another and from all contaminations, especially useful when the spores are exceedingly small. It was developed and used by the writer at Iowa State College in 1916–17.[7] Later Durrell (*8*) used the same method for making single spore isolations. Many workers have used the Van Tieghem cell for isolating single spores of fungi, as, for example, Sherbakoff in his work on the genus Fusarium (*12, p. 102–104*), in which he emphasizes the importance of obtaining single spore strains and the difficulties often attendant thereupon; and most noteworthy of all, Emil Christopher Hansen who as long ago as the middle of the nineteenth century used a method very similar to the one here described for securing single yeast cells in fermentation technic (*11, p. 106, 107*). But insofar as the present writer has been able to determine, precisely this method has not previously been described. When spores are large enough to be seen with the low power through the bottom of a Petri dish and contaminations are not a serious factor, this method would not be justified, of course.

The several cultures derived from all of these three sources of ascospores were typical in every way of the Botrytis cultures obtained from conidia. The typical gray mold developed quickly, and this was followed shortly after by sclerotia. Inoculations of castor bean plants made with these cultures, after the usual method (described on p. 703). produced the typical disease. Reisolations made from such diseased plants were held and used, along with numerous other cultures, for later production of apothecia.

[7] GODFREY, G. H. CULTURAL STUDIES OF LEPTOSPHAERIA CONIOTHYRIUM CAUSING RASPBERRY CANE BLIGHT. Thesis, Iowa State College, 1917. (Not published.)

DEVELOPMENT OF APOTHECIA UNDER ARTIFICIAL CONDITIONS

Immediately upon receiving the intimation of the existence of a perfect stage of this organism the writer took steps to work out a means of producing apothecia under controlled conditions. In view of the fact that the only apothecia found at first were from tissue transfer cultures (isolation record, p. 688, B 1 and B 21), it was thought that the fungus might be heterothallic. Consequently several cultures were made consisting of mixtures of pure-line strains in various combinations of single-conidium, single-ascospore, and tissue-fragment cultures. No differences were noted in the development of sclerotia; and, as soon became evident, there were no differences in apothecium production. A number of other experiments were started, consisting principally of subjecting sclerotia to different conditions with the idea of determining their effect on apothecium production. An account of these experiments follows.

EXPERIMENT III.—March 12. Some sclerotia from the field, one of them large, widespread, and flat, were placed on a layer of moist sand on a glass plate, and the whole was covered with a battery jar. This was placed on the laboratory table near a window. It was watched carefully for several days without developments, then, in the stress of other work, was neglected for some time. During this period the sand became quite dry. Notwithstanding this, on April 16 five apothecial stalks were seen to have risen from the large flat sclerotium. One was about 1 cm. long and 1½ mm. wide at the end, all the others being not so fully developed. The sand was at once moistened before the apothecia had suffered materially. These will be designated lot III A. At the same time additional sclerotia, which we shall call lot III B, were incubated as follows:

Sclerotia from the field.
Sclerotia from strain B 21 B (1).
Sclerotia from strain B 21 C (a) 1, 4, and 7.

These sclerotia were placed on moist sand in well-marked positions and covered with a battery jar, under laboratory conditions.

Observations were recorded as follows:

Lot III A. April 24. The largest apothecium, already noted, is developing very irregularly. The second has a perfect disk 1½ mm. in diam. The third, half as large, has a hollow end. The fourth is somewhat shriveled, and the fifth is growing.

April 26. The first apothecium in its irregular growth is developing two smaller distinct disks on the side of the first.

April 29. Early in the morning the bell jar was lifted, and the second apothecium was observed to shoot forth ascospores in a distinct cloud. April 30, the bell jar was lifted again, and the spores when ejected were caught on a plate of clear corn-meal agar. Single ascospore isolations were made. Apothecia I and II of this lot were photographed and later preserved in alcohol. The third was sent to Prof. Whetzel at Cornell University.

May 9. A dozen or so more apothecia, well developed, were noted from this lot.

Lot III B. May 6. Apothecial stalks are developing from both field and pure culture sclerotia.

May 10. One or two apothecia from the field sclerotia are nearly mature.

May 15. An apothecium from a sclerotium developed in tube B 21 C (a) 4 is mature, as shown by the appearance of a cloud of spores when the cover was lifted. This particular group of sclerotia was isolated from the others and covered separately.

May 16. The cover was lifted and ejected spores were caught on a poured plate of corn-meal agar.

May 17. Another "volley" was caught, the first being too thickly sown. Single spore isolations were made, these therefore being second-generation ascospore cultures. That is, starting with an ascospore, the culture has gone through its entire life history, gray mold, sclerotium, apothecium, and a new generation started with an ascospore.

Experiment III demonstrates that under favorable conditions of moisture and temperature apothecia can be produced extensively. It also shows that single ascospore strains of the fungus are in themselves capable of producing all the stages of the life history of the fungus—that is, the fungus is purely homothallic.

EXPERIMENT IV.—March 18. In an attempt to influence apothecium production in pure culture, strains 3, 5, 7, 9, 12, 13, and 14 were transferred to new tubes, and the originals, all containing an abundance of sclerotia were submitted to the following conditions:

No. 3 was placed at once in a moist chamber at room temperature.

No. 5 in ice box for 48 hours at 10° to 13° C., then as in No. 3.

No. 7 above a freezing mixture starting at −10° C., overnight, in a well insulated fireless cooker, then as in No. 3.

No. 9 above a freezing mixture overnight, then as in No. 3.

No. 12 in ice box for 14 hours, than as in No. 3.

No. 12 2d in freezing mixture overnight, then as in No. 3.

No. 13 in freezing mixture, with gradual rise to +10° C., 36 hours in all, then as in No. 3.

No. 14 in freezing mixture overnight, then as in No. 3.

The moist chamber was on a shelf in the laboratory, some distance from any window. The cultures were watched for several weeks. No development of apothecia occurred on any of them under those conditions. Finally they were removed from the moist chamber and placed in a culture-tube rack on the laboratory shelf.

Eventually, later in the summer, several sclerotia from these tubes gave rise to apothecial stalks. Included in those so doing were tubes Nos. 9 and 13, which showed that the actual freezing of the sclerotia was in no way injurious to the life of the fungus. Neither did it stimulate apothecium production in any way.

EXPERIMENT V.—April 12. A large number of field sclerotia were placed in Petri dishes of unsterilized moist sand. These were subjected to varying conditions, particularly with regard to temperature. Plate No. 6 placed in a moist chamber at room temperature was the first (May 10) to start to develop apothecia. No. 4 was held until May 24 in a moist chamber in a warm dark room without development of apothecia and on this date was moved out of doors, still maintaining moist chamber conditions. Almost immediately thereafter apothecia began to develop.

Nos. 2 and 5, held in the ice box until May 26, were on this date placed out of doors with No. 4; and also shortly afterward they developed apothecia. On May 26 plates 3 and 6, the former having been held for the first week in the ice box, each had a dozen or so beautiful apothecia.

From this experiment it would appear that high humidity and a warm temperature are the primary requisites for apothecium production. There are strong indications that light as well is an essential factor.

EXPERIMENT VI.—April 15. Several large, black sclerotia from various pure culture tubes were picked out under sterile conditions and placed half buried in sterile sand in Petri dishes. These were subjected to varying temperature conditions as in experiment V. Here, too, the cooling in the ice box and alternate cooling and warming had no beneficial effect, for apothecia were first produced on the plate placed promptly under a bell jar under room temperature conditions. On May 10 sclerotia from strain B 11 showed two large apothecial stalks developing. On May 26, in addition to mature apothecia from B 11 they were found also from strains B 1, B 19, B 21, and B 21C.

In this case mature apothecia were produced from definite single conidium strains of the organism, namely B 11, and B 19, under sterile conditions.

EXPERIMENT VII.—April 17. A large number of sclerotia from castor-bean inflorescences and stalks in the field were placed in sterile sand in Petri dishes and subjected to varying temperature. As in experiments V and VI there was no particular difference in apothecium production in the different lots. On May 10, plate 1, placed at once under warm, moist conditions, showed many stalks arising. Plate 2, placed first in the ice box for a week and then placed with No. 1, also showed several. Plate 3, in a covered Petri dish, showed two or three on May 17. Evidently the lack of aeration had inhibited their development to some extent. On May 26, Nos. 1 and 2 showed dozens of mature apothecia, which were used to swell the collection. No. 3 as well showed several mature apothecia.

At the same time that these sclerotia were started, others were surface sterilized by momentary immersion in 95 per cent alcohol, and then for 3 minutes in 1 to 1,000 mercuric chlorid followed by two changes of sterile water, then placed on a corn meal

agar plate, and on sterile sand. On April 23 one sclerotium was developing conidia; on May 10 conidia were developing from most of the sclerotia; and on May 26, a sclerotium on the agar was developing an apothecium.

Thus, under sterile conditions, the apothecial stage was produced from field-borne sclerotia.

EXPERIMENT VIII.—April 15. A large number of sclerotia from the field, mostly from Dr. Howell's place at Orlando, Fla., were placed on moist sand in half-liter Erlenmeyer flasks and subjected to a variety of conditions.

May 8. Flask No. 1, placed at once in a moist chamber in the laboratory, showed apothecial stalks arising from two or three sclerotia. Flask 2, which had been placed out of doors under the east side of the building, where it was subject to a little sunlight early in the morning, and during the day to ordinary daylight conditions, with a temperature fluctuating from rather cool at night (approximately 10° C.) to rather warm in the day (approximately 27°) showed several small apothecial stalks arising. Flask 4, after having been held in the ice box for a week, then subjected to room conditions, showed dozens of stalks, none of them as yet mature.

May 12. Flask No. 1 showed many well-formed apothecia. No. 2 showed more than a dozen short stalks with well-formed cups nearly mature. No. 4 showed many well-formed cups. On May 22 these were photographed within the flask (Pl. 11, B), and then removed still attached to the sclerotia and photographed in a Petri dish. (10, Pl. XL). Some of these were then dried in the sun, and others were preserved in alcohol.

May. 24. Flask No. 3, which had been held thus far in the dark under conditions as favorable as any of the others as regards moisture, and in a constant warm temperature, without a sign of the development of apothecia, was placed out of doors under the east side of the house. Almost immediately apothecial development began to occur. On June 2, eight days later, several apothecia as well formed as in No. 2 had arisen.

This experiment, together with the repeated observation that had been made that apothecial stalks were distinctly phototropic, indicated that light is one of the essential factors for the production of apothecia from sclerotia. It also indicated that the use of moist sand in Erlenmeyer flasks was a convenient and favorable method for keeping moisture conditions right. Thereafter this method was used extensively for the production of apothecia.

EXPERIMENT IX.—May 28, 1919. A large number of field sclerotia were collected and planted upon moist sand on a glass plate placed upon the laboratory table, the whole being covered with a bell jar. On June 3, six days later, apothecial stalks are already arising.

The development being so much quicker in this case than ever before indicates a marked seasonal influence. Whether this consists of simply more favorable temperature or of a difference in light duration or of some other factor is not known.

A still later placing of sclerotia, these under absolutely pure culture conditions, the sclerotia having been derived from pure cultures and having been placed on sterile sand, was examined on September 17. Apothecia were produced in abundance from sclerotia of strains B 1, B 21, B 35, and B 37, the two latter having been authentic single conidium strains.

EXPERIMENT X.—About the middle of March field sclerotia were collected and subjected to outdoor conditions in a variety of locations in an attempt to produce apothecia under more nearly natural conditions. The first lot of sclerotia was placed on sand in a shallow wooden box, which was subjected to moist conditions by placing on a thick bed of "Spanish moss" (*Tillandsia* sp.) kept saturated with water, and covering the whole with a large moist chamber built of window sash. On April 21 it was noted that this was becoming very hot during the middle of the day, and since apothecia had not yet been produced, the box of sand was removed and placed in the shade of some boxes on the west side of the house and kept moist by waterings from time to time. On April 24 under these more favorable conditions, temperature apparently being a factor, apothecial stalks began to form, and on May 3 mature apothecia were collected.

Lot 2 was placed on sand in a small shallow box which was sunken till the top was flush with the ground under the east edge of the laboratory. It was watered occasionally, but at times the sand was quite dry. Finally, on May 10, a few very small apothecia were found.

Lot 3. On April 15 field sclerotia were placed in marked locations in a castor bean field under natural conditions. On June 2, after several days of continuously wet weather, a few apothecia were found to have developed.

It would seem that a moist substratum alone is not sufficient but that also a moist atmosphere is necessary for the best production of apothecia. It would also seem that too high temperature is capable of inhibiting the development of apothecia.

EXPERIMENT XI.—It has been noted in experiment III that single ascospore cultures from second-generation apothecia had been made. The development of sclerotia was so prompt in these cultures that it seemed possible to place these sclerotia under favorable conditions and get still another and perhaps several more generations. Early in July, 1919, this was done. Before the end of the month a third generation of apothecia was produced. A mature apothecium was laid on its side in a poured plate of clear agar and left several hours. When the cover was lifted the apothecium shot forth a cloud of spores, which were, of course, caught on the surface of the agar. Single ascospores were isolated and further cultures started. These, too, produced sclerotia which produced another crop of apothecia, this time within 30 days of the time the single ascospores were isolated. Thereafter this process was continued, at first in Florida and later in Washington, D. C., whenever time permitted, in some cases after delays of several months. In July, 1920, the eighth consecutive generation of apothecia was produced, but there was then no opportunity to secure single ascospore isolations. This was accomplished in August. At the same time, following a suggestion from Prof. W. B. Brierley, of the Institute of Phytopathological Research, Harpenden, England, who was then a visitor in America, single conidia taken from the seventh-generation single ascospore cultures were isolated to determine whether they, too, could develop the perfect stage. These cultures were placed in racks in the laboratory. After three months' absence, the writer returned and found that many of the tube cultures, both single ascospore and single conidium, had produced apothecia in the tubes. These, however, had shriveled and become covered with conidia and mycelium. Consequently in November new single conidium isolations were made. In addition, castor bean plants in the greenhouse were inoculated with the seventh-generation strain, the typical disease was produced, and single conidium isolations were made, directly from the host. At this time, also, mature eighth-generation apothecia were available, and single ascospore isolations were made from them. Transfers of all three strains were made to several tubes of oatmeal agar, and in due time an abundance of sclerotia developed. On December 15, several sclerotia were transferred from each lot to Erlenmeyer flasks of moist sand. In addition, sclerotia that had been collected in the field at Orlando, Fla., in July, 1919, were placed under like conditions. All the flasks were placed in the greenhouse. After several weeks an examination disclosed that every one of the four strains had borne mature apothecia.

Thus ninth-generation apothecia were procured, each generation after the first having started from a single ascospore; single conidium strains from two sources both produced normal apothecia; and field sclerotia a year and a half old did likewise. All four strains were used to start new single ascospore cultures according to the usual method. Plate 10, C and D, were taken from two of these plates.

Eventually tenth-generation apothecia were developed, and ascospores were sown and isolated from them. In the entire series there appeared to be not the slightest change in appearance in culture, as compared with more recent isolations, or in ability to develop all the life-history stages.

INFLUENCE OF LIGHT ON APOTHECIUM DEVELOPMENT

In all this work the conclusion could not be escaped that the production of apothecia was much more readily accomplished in the summer months than in the winter. It was very rarely that apothecia were

produced in the culture tubes themselves in the winter time, whereas in both the summers of 1919 and 1920 this phenomenon occurred regularly. That temperature was one factor involved in this seems evident, for the observation was made repeatedly that at the lower temperatures, even with good light and moisture, apothecium production was slow and the stalks were abnormally thin and the cups not well opened out. It was evident as well that light was involved. Preliminary experiments were started in November, 1920, to investigate this point further.

EXPERIMENT XII.—November, 1920. Mature sclerotia were taken from several different tubes and evenly divided between two 250-cc. Erlenmeyer flasks, each containing 100 gm. of sterile sand and 25 cc. water. Thus the conditions were as near alike as possible. One of the flasks was then wrapped completely in black paper. Both were placed under a bell jar on the laboratory table with a dish of water beneath in order to keep the humidity high. A 60-watt Mazda light was placed about 18 inches to one side and was kept lighted every night for several weeks. The nearness of the light was adjusted until the temperature within the bell jar was about 25° C., which had been shown to be the optimum for development of the fungus. For a long time no development occurred. After two months, however, numerous apothecial stalks commenced to rise from the sclerotia in the flask exposed to the light. These attained considerable length, in some cases at least 2 cm., and all leaned very strongly toward the source of light. None of them opened out into normal apothecia, however. In the dark flask no development whatever occurred.

EXPERIMENT XIII.—December, 1920. After this very clear demonstration of the influence of light was observed, a further, more extensive series was started, with the cooperation of Mr. H. A. Allard, who very kindly consented to the use of his controlled light conditions (*9*) in the greenhouse at Arlington Farm. This was primarily a length-of-day experiment, and involved the turning on of electric lights at dusk in the evening, leaving them on long enough to make the day the length desired. Lights of different intensities were used, the intensity being determined by the nearness of the light to the subjects of the experiment. Two flasks each, prepared as described for experiment XII, were placed under four different conditions: (1) a 20-hour day, the source of added light being small Mazda lamps near the roof of the greenhouse several feet away from the flasks; (2) a 20-hour day, the added light being a 100-watt lamp about a foot distant from the flasks; (3) a 20-hour day, with a 100-watt lamp about 3 feet distant; (4) the normal day for that time of year, approximately 9 hours. In addition, a flask prepared like the others in all respects was covered completely with black paper to shut out all light. Temperature and moisture conditions were as nearly alike as could be maintained under ordinary greenhouse management. The sclerotia in the different lots were from identical sources, and included some from nature as well as from artificial cultures. Observations were made once a week, and for three weeks no change was to be seen in any of the flasks. On the fourth week one flask of lot 3 showed early stages of apothecial formation. Six weeks after the experiment was started mature apothecia were found in all the flasks subject to increased day length, the largest and best specimens being found in lots 2 and 3, while in the flask under ordinary daylight conditions a few spindling stalks had arisen, which never opened out. The sclerotia in the dark container had not developed the slightest suggestion of an apothecial stalk.

This experiment again shows the influence of light on the germination of sclerotia, but results are still far from satisfactory. The normal summer development of apothecia was not even approached under the artificial conditions that prevailed. It is to be hoped that some more work can be done along this line.

PERFECT STAGE IN NATURE

The first appearance of the perfect stage under altogether natural conditions was not observed until early in July, 1919. After a prolonged rainy period at Orlando, Fla., a careful examination was made of castor-bean plants and of the ground beneath in the Boardman field. Several apothecia were found arising from sclerotia that had fallen to the ground.

In addition, several castor-bean stalks were found (July 21 and Aug. 1) with sclerotia still attached producing apothecia in great abundance (Pl. 6, D). It has been seen that each apothecium is capable of discharging spores in a distinctly visible cloud. It is clear, therefore, that, even were the conidial stage not capable of living over the winter, the apothecial stage thus produced would be capable of starting an abundance of primary infections.

CONCLUSIONS FROM EXPERIMENTS

These experiments and observations bring out the following points of interest in connection with the development of apothecia of this organism under controlled conditions:

(1) Moisture. Reasonably moist conditions must prevail, both in the substratum upon which the sclerotia are resting and in the air above.

(2) Temperature. While a wide range of temperature will permit of their development, most rapid growth occurs under fairly warm conditions, averaging about 25° C., the optimum for the vegetative growth of the organism.

(3) Aeration. Provision must be made for access of air to the sclerotia; too small an air space above them, as for instance that which would be found in a covered Petri dish is not as favorable as a greater amount, other things being equal.

(4) Light appears to be necessary, both as an initial stimulus and for continued normal development; those developed with insufficient light are long, slender, and irregular and do not open up normally.

(5) Subjecting sclerotia to freezing, drying, or an extended dormant period previous to placing them under favorable conditions does not favor apothecial development.

(6) The fungus is purely homothallic. Sclerotia from single spore cultures develop apothecia as readily as do those from mixed strains.

MORPHOLOGY AND PHYSIOLOGY OF THE PERFECT STAGE

It is hard to distinguish the apothecium macroscopically from that of any other Sclerotinia. It is smaller, on the whole, than those of the well-known forms *Sclerotinia libertiana* and *S. cinerea*, the largest seen having a disk only 7 mm. in diameter; the stalk normally is only 1 or 2 cm. long. The disk or cup is not so often funnel-shaped as it is with the other two forms, but it is more frequently opened completely or even recurved, as shown in Plate 11, C, and in Plate XL of the writer's earlier paper (*10*). The color is that of most Sclerotinias and varies somewhat from cinnamon brown to chestnut brown, the stalk sometimes being lighter, sometimes darker. From one to several may be borne upon a single sclerotium.

Any unfavorable condition that may arise while apothecia are forming may cause strange abnormalities in their structure. They often fail entirely to open out, the tip being tubular for a short distance back. Such forms have no normal asci, but rather hyphal tips resembling the ordinary paraphyses. Another abnormality that has been observed after a temporary check in the growth of an apothecium has been a division near the tip into several distinct cups. Sometimes these have failed to mature, and again they have developed several normal mature ascus producing disks. Plate 11, D, shows an apothecial stalk with five distinct disks.

The discharge of spores from an apothecium was repeatedly observed. This appears to be brought about by a sudden change in the humidity surrounding them. When a good "crop" of apothecia have reached maturity in one of the flasks, as shown in Plate 11, B, or in any other moist chamber in which they have developed, opening the chamber to the air by removing the stopper or otherwise will result in the sudden discharge of a cloud of spores. This phenomenon may not be observed again until the chamber has been closed for several hours and again opened, when it will be repeated.

Many ascospore isolations were made by placing a mature apothecium on its side on a poured plate of clear agar with the disk facing the center. When the apothecium is left there for a few hours, the removal of the cover causes the sudden discharge of ascospores, which may be carried the full width of the plate. After such an occurrence the deposit of spores can sometimes be clearly seen with the naked eye on the surface of the agar, so thick are they. Microscopic examination of one such plate disclosed the interesting fact that often all eight spores from a single ascus when discharged clung together as if in a gelatinous matrix. Large areas on the plate were covered with groups of eight spores. Plate 10, D, is a photomicrograph taken with a Zeiss 2.5-mm. water-immersion objective placed directly over one of these areas on the plate. Ascospore discharge also takes place either intermittently or the spores may be scattered when ejected, for spores may be found on the plate either singly or in groups of two or three. By examining the plate through the bottom with a low-power objective near the edge of the wedge-shaped area in front of the disks, it is an easy matter to locate as many spores as may be desired and mark them for transfer to another plate or a tube.

Ascospore germination takes place very quickly on a plate of agar. Normally it is by the production of a germ tube which continues to grow vegetatively until conidiophores arise, and the gray mold stage is formed, which is usually within 36 hours. In one case, when such a plate as the one just described was held in the ice box over night, it was observed that microconidia were being produced on the ends and sides of comparatively short germ tubes. This condition is pictured in Plate 10, C. The microconidia continued to develop during the day, a succession of them being borne on the end of a single conidiophore, until quite a group of them were to be seen. In no case could germination of microconidia be seen. The same plate shows normal germination of ascospores by germ tubes. This is also shown in various stages in Plate 12, H and I, which consists of camera-lucida drawings of spores from which the first ascospore cultures were derived.

DESCRIPTION OF THE FUNGUS

For the convenience of the reader the complete description of the fungus, as published in 1919 (*10*), is repeated here.

SCLEROTINIA RICINI

Apothecia one to several from a single sclerotium, 5 to 30 mm. high, usually 6 to 15 mm., infundibuliform to cyathiform and discoid, long stipitate, cinnamon brown to chestnut brown; stalk concolorous, cylindrical, slender, smooth, flexuous, attenuated below, without rhizoids; disc at first closed, expanding to saucer-shaped with margin sometimes recurved, exterior roughened, 1 to 7 mm. in diameter, usually 1.5 to 4 mm.; asci cylindrical to cylindro-clavate, apex slightly thickened, opening by

a pore, 50 to 110 μ by 6 to 10 μ, usually 80 to 100 μ by 8 μ; ascospores 8, ellipsoidal, often sub-fusoid, hyaline, continuous, bi-guttulate, 9 to 12 μ by 4 to 5 μ; paraphyses abundant, filiform, septate, hyaline, 1.5 to 2 μ in diameter. Conidial stage (*Botrytis* sp.) forming widespreading cobwebby or somewhat woolly mass, pale drab-gray to drab, dried specimens dark olive-gray; sterile hyphæ procumbent, hyaline, many-septate, often vacuolate, frequently anastomosing; fertile hyphæ long, slender, smooth, slightly constricted at the base, olivaceous when mature, dichotomously branched, terminal branching compact, apices noninflated, thin-walled, collapsing when the conidia fall; proliferation sometimes occurring; conidia borne on sterigmata, globose, smooth, hyaline, 6 to 12 μ, usually 7 to 10 μ, compactly grouped; microconidia globose, hyaline, 2 to 3.5 μ, borne apically on short, obclavate, single or clustered conidiophores that develop on the sides of hyphæ or on tips of special branches; appressoria rare, microscopic, 20 to 60 μ across base. Sclerotia black, rough, elongate, irregular, 1 to 25 mm. in length, usually 3 to 9 mm., suberumpent to superficial, often anastomosing, developing on axes and peduncles of old castor bean inflorescences and on stalks.

Parasitic on *Ricinus communis*, usually on inflorescences, also on stems and leaves; type locality, Orlando, Fla.; distribution Florida, Mississippi, Louisiana, Texas, and Cuba. Type specimens deposited with the Office of Pathological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

ORIGIN OF THE PATHOGENE

Early in the progress of the disease it was suspected that the pathogene had been introduced with the seed. This was not proved absolutely for some time, but much evidence pointed in that direction. For example, the disease was found in nearly every field where Bombay seed had been planted, provided the climatic conditions were favorable for the development of the fungus. One such field was at Baton Rouge, La. A field planted to American-grown seed only a mile or two away was not attacked. Native castor beans had been grown for years in Florida without any report of it, and in 1918 they were not affected until late in the season. Much evidence was found in connection with seed produced in diseased fields in America. The fungus was capable of living over for a long period on the seed. On October 30, 1918, specimens were collected at Alvin, Tex. The fungus was readily isolated, and some of the material was laid away in a sealed envelope. Two months later the fungus was again isolated from this same material. On October 25, waste from a hulling plant at Plant City, Fla., including some light-weight beans, was collected. Two weeks at least must have elapsed since the beans were harvested. The material was placed in a sealed envelope. Six months later the fungus was isolated from this material. Plate 7, D, shows the development of the fungus on one of the castor beans in a poured plate of nutrient agar. Finally, sclerotia 17 months old developed the apothecial stage. This shows that sclerotia carried with the seed are able to reproduce the disease even after a long period.

In addition, many observations showed the close contact of the fungus with the developing seed pods in diseased spikes. A castor bean reaches full size long before it is matured. It is then soft and very succulent and the caruncle is prominent, as shown in Plate 7, A. In such condition it offers no resistance to the penetration of the fungus. Plate 7, B, shows a seed in its pod with the mold growing out of the caruncle and even the seed coat; C shows several such seed with the gray mold distinctly visible; E shows several seed which had been attacked before maturity, with sclerotia attached to the seed coat and the remains of the pods. These specimens were collected under natural conditions in diseased castor bean fields.

The writer did not have an opportunity to study the original imported seed until well into the winter. Then repeated attempts were made to isolate the organism, without success. H. E. Stevens, of the Florida Experiment Station, however, working on this phase of the problem earlier in the fall, succeeded in isolating the causal organism from samples of the imported seed. (Letter of May 9, 1919.) He planted them on sterilized sand, and on a few seed the gray mold developed. Later, the writer made a final examination of some of the Bombay seed in one of the original bags, and a single small sclerotium was found. This was planted on a plate of moist sand under a bell jar, and eventually an apothecium, the perfect stage of the fungus, developed.

Letters written to pathologists in India were at first not completely satisfactory in leading to the solution of the origin of the fungus, the earlier replies stating that it was not known there. A subsequent letter from J. F. Dastur, dated February 15, 1921, brought word that the disease had been found in Mysore, South India, and was under investigation by one of the plant pathologists. More recently (Aug. 13, 1921) a culture was received from India. This culture contained only conidia, which in form and size appeared to be identical with the American form. Later appearances in culture, however, throw some doubt on its identity with our organism. In oatmeal agar the sclerotia are not so abundant, conidia are fewer, conidiophores not so definitely dichotomous, and the development of white mycelium is greater. No perfect stage has developed for final comparison. In spite of this possible discrepancy, the evidence is very strong that the pathogene came from India to this country along with the seed. The fact that it has been discovered there only recently, if at all, is especially interesting, in that it appears to present one more case of an introduced parasitic fungus being more serious in its new habitat than in its old.

INOCULATION EXPERIMENTS

Field observations made the first year, in the course of surveys in the South, made it clear that for successful inoculations a prime requisite would be moisture. After a few preliminary inoculations it became evident that an abundance of moisture was necessary not only for primary infection to take place but for the disease to develop in the host, even after the fungus had penetrated into the interior. The first inoculations were made on seedlings by applying the fungus along with some of the culture medium to different parts of the plant, without injury, and then covering with a bell jar. This resulted almost invariably in success. Within 24 hours positive infections became evident. Plate 8, B, shows distinct lesions on a young seedling, produced by artificial inoculation in the Cornell University plant pathological greenhouse, in January, 1919. Other inoculations made at the same time consisted in the application of conidia to young growing leaves. These were successful unless the plants became dry during the incubation period of the fungus. Material of plants, particularly leaves, thus infected, was fixed for later histological work to determine the manner of penetration of the fungus into the host. Castor bean seeds were also sterilized in calcium hypochlorite, after the method described by Wilson (*23*), and planted in large 10-inch tubes of synthetic agar, with the result that about a dozen castor bean plants in pure culture were secured. Inoculations were later made on these with resulting rapid infections.

Most of the inoculations were made, however, at Orlando, Fla., on developing inflorescences of large growing plants. Since the nature of the plant demanded special methods, these will be given in detail. The method most used was one obtained from J. R. Winston, who used it extensively in his work on citrus diseases. For an inoculation chamber a sheet of ordinary waxed paper was used. First a wad of cotton was wetted and wound about the base of the inflorescence to supply moisture. Then the inoculum was applied in the particular form to be tested, and the whole was promptly covered with the waxed paper in the manner depicted in Plate 8, A. First, the sheet was folded lengthwise about the stem, and the vertical edges were brought together and folded twice with narrow folds and creased firmly. Then the upper part was folded down and brought around the stem and held tightly in place with one or two ordinary paper clips. In this way a tight moist chamber was secured in a very short time. It was thrown away at the end of the desired incubation period.

For inoculations with ascospores, it was necessary to be absolutely sure that no conidia were present. A few preliminary tests on plants under natural conditions made it evident that it would be difficult with ordinary methods to insure sterility of parts of the plant to be inoculated. A waxy bloom covered practically the whole plant, and particularly the inflorescence and prevented the spread of solutions of mercuric chlorid applied for the purpose of sterilizing the surface. This difficulty was overcome by using this sterilizing agent in solution in 35 or 40 per cent alcohol. Immediate and complete contact was obtained in this way, and it was determined that 20 seconds' immersion would not injure the plant. This treatment was followed immediately by dipping the part treated, usually the inflorescence, into freshly drawn tap water, which removed the alcohol and mercuric chlorid, and left the plant evenly wetted, an additional advantage when it came to applying the inoculum.

In May, 1919, no natural infection had as yet become evident in the experimental plantings near the laboratory at Orlando, Fla., where inoculations were made. Conditions were the better, therefore, for reliable results. A series of 10 inoculations, with an equal number of controls, was made on castor bean spikes in different stages of development after the methods outlined above. The plants were not sterilized. Conidia from young cultures in Petri dishes were used for most of the inoculations. Transfers containing mycelium and some of the culture medium were used in others. Eight of the 10 inoculated flower clusters showed, at the end of 48 hours, definite signs of infection. The two failures had dried out, owing to insufficient sealing of the paper moist chamber. None of the controls were infected. Typical inoculated inflorescences, removed from the plants and placed in flasks of water under bell jars in the laboratory, developed a heavy growth of gray mold very similar to its appearance in nature. Those left exposed, out of doors, failed to progress. The rainy season had not yet started sufficiently to develop and spread the disease.

Several more inoculations were made at this time, with different strains of the organism, including reisolations from previous inoculations and cultures derived from ascospores. Practically all of them were successful, and the controls remained healthy.

On May 20, inoculations were made with ascospores as follows: Several apothecia were crushed in sterile water and the resulting suspension of ascospores, asci, etc., were applied to castor bean inflorescences not pre-

viously sterilized in small drops with a platinum loop and in considerable quantities by pouring it into the spike. Seven inoculations were made. Two only were successful, those in which the inoculum was applied by pouring. Isolations made from the infected spots produced typical Botrytis in pure culture. On May 24 a further ascospore inoculation was made. A potted plant was secured, the large interfering leaves were removed, and a half-grown inflorescence was sprayed with sterile water. An Erlenmeyer flask containing a large number of mature apothecia was now taken, the stopper was removed, and the resulting cloud of discharged ascospores was blown, with the aid of a rubber tube which had been immediately inserted into the flask, upon the wet spike, which undoubtedly caught thousands of them. The inflorescence was then covered in the usual way for 48 hours. On May 26 infection was evident on two or three male buds. One of them was removed and the surface sterilized, then used to inoculate a poured plate of agar, with a resulting development of Botrytis stage of *Sclerotinia ricini*. The inoculated spike was then cut from the plant and placed in a moist chamber. In three or four days a copious development of the typical gray mold occurred. (See Pl. 3, C.)

The writer was absent from the station during the month of June, and when he returned in July all his experimental plantings were scatteringly infected. This naturally made any further inoculations more or less uncertain, for it was impossible to determine in advance whether or not an apparently clean spike had been previously infected. However, early in September a few more ascospore inoculations were made, this time by placing well-developed apothecia directly into the sterilized and washed inflorescences in marked locations, holding them in place with paste and covering with waxed paper. Six cases out of 10 inoculated showed infection after the usual two days, traceable apparently to the apothecia. Two showed infection appearing elsewhere than near the applied apothecia, and 2 out of 6 controls were infected. The results of these inoculations therefore would be more or less unreliable were it not for the fact that subsequent examination showed penetrating hyphæ directly traceable to germinating ascospores.

At this time also a considerable number of spikes were inoculated with conidia, and material was fixed at different periods for histological study.

HISTOLOGICAL STUDIES

It has been mentioned in various parts of this paper that materials were fixed and embedded for later histological work. The first consideration was to determine the manner of host penetration. A large amount of material was secured for this purpose, both from seedling inoculations at Cornell University, Ithaca, N. Y., and from field inoculations at Orlando, Fla. Material was fixed at different periods after spore inoculations in Fleming's weak killing fluid. The different lots of material were washed and run up to 70 per cent alcohol in the field and held there for several months, then embedded in paraffin at Washington, D. C., during the winter of 1919–20. Several different kinds of differential stains were tried, but best results were secured with Pianeze IIIb (*21*) and with Fleming's triple stain.

Early stages of penetration appeared to be practically identical with those pictured by Blackman and Welsford (*1*) for *Botrytis cinerea*. The leaves of plants grown in pure culture in 10-inch culture tubes on synthetic agar were inoculated by applying conidia thickly on the surface. Mate-

rial fixed 24 hours after inoculation showed abundant spore germination and early stages of penetration before there was any indication of injury to the parts inoculated. Actual penetration of the cuticle was accomplished, as in the case of *B. cinerea*, by a fine peglike projection from the point of contact of the developing germ tube. Plate 13, A to H, shows camera-lucida drawings of 5 to 7 μ sections of castor bean leaves, showing hyphae penetrating the cuticle. No attempt was made to demonstrate muscilaginous substance at the point of contact of the germ tube. After penetration of the cuticle was accomplished, the fungus advanced quickly into the host, and breakdown and disorganization of the host tissue was rapid and complete. The cuticle remained a barrier to easy penetration of other hyphae for some time, however. Sections of materials fixed 48 hours after inoculation showed a tangled web of germ tubes, with abundant production of appressoria and distinct indentations below many of them, indicating marked mechanical pressure. Plate 9, A and B, shows such indentations. The disorganization of the host tissue beneath, with consequent loss of turgor, has permitted deeper indentation than occurs with initial penetration. The tangled web of hyphae of germinating spores which is very evident in the figures would seem to be in itself a factor in withstanding the backward pressure exerted by a penetrating tip. These sections were very thick, approximately 25 μ, and were photographed to show the abundance of hyphae and their apparent pressure on the host, in this case a male blossom bud. Plate 9, C, shows hyphae of the fungus within such a male blossom bud and the complete disorganization of host tissue.

Sections of nectaries from a castor-bean inflorescence showed the protective cuticle to be continuous over its entire surface. The cuticle appeared in some cases to be even thicker over the nectary than over the surrounding parts of the host, but there is no evidence for a conclusion that this had any influence on the comparative freedom of nectaries from primary infections.

The fact that the seeds are important carriers of the pathogene has already been mentioned. Many observations were made of the presence of the mold on and within the seed. Plate 7, B and C, shows the mold itself growing on the caruncle of castor bean seed. The mold was often seen growing over immature pods and penetrating them. Such materials were fixed and embedded, and the presence of hyphae of the fungus was demonstrated with differential stains. Since such seed may reach the size and appearance of maturity, it is evident that the fungus may be carried by this means, along with healthy seed.

HOST LIMITATIONS OF THE FUNGUS

One of the first considerations, when it was determined that the gray mold was a new organism in America, was to determine whether or not it might prove to be a danger to other crops. At Cornell University, in January, 1919, a number of plants that were available were inoculated under exactly the same conditions that were given the castor beans inoculated successfully at the same time. The common geranium (*Pelargonium* sp.) was more severely infected than any of the others. The growing point of the plant was entirely killed, and several of the younger leaves were attacked at their margin (Pl. 8, C). The organism continued to spread as long as moisture conditions were kept favorable. Reisolations were made and identified. As soon as the moist inoculation

chamber was removed the disease stopped progressing, as it did with the castor bean plants. Besides the geranium, eggplant (*Solanum melongena* Linn.) seedlings became very slightly infected. Among the plants that were entirely unaffected by the organism were tomato, onion, snapdragon, sunflower, string bean, tobacco, and lettuce.

In Florida, during the season most favorable for infection, a few other plants belonging to the Euphorbiaceæ, the family to which the castor bean belongs, were inoculated under the same conditions as those given castor beans. Plants inoculated were five or six each of Jatropha (*Jatropha* sp.), Cassava (*Manihot ultilissima* Pohl.), and Poinsettia (*Euphorbia pulcherrima* Willd.). Slight but definite and characteristic lesions were produced on all of these, but in no case did the organism spread sufficiently to indicate that it might ever become active as a parasite on any of them. At the same time geranium plants were inoculated, and with similar results. All the lesions dried up after moist chambers were removed, even though castor beans in the field were meanwhile actively attacked.

Throughout the summers of 1918 and 1919, during the course of survey trips through the various infected localities, a close watch was kept on all manner of vegetation in the vicinity of diseased castor bean fields for possible natural infection. Other than castor beans no diseased plants were found for which the castor bean gray mold was responsible. The writer considers it safe to state that in his opinion the gray mold organism, fatal as it is to the castor bean crop, need not be considered a menace to any other crop. It appears to be very narrow in its host limitations.

VARIETAL SUSCEPTIBILITY

In order to make a test for possible varietal resistance, a collection of as many different varieties of castor beans as possible was made during the winter of 1918–19. These consisted principally of varieties grown by the Office of Drug and Poisonous Plant Investigations of the Bureau of Plant Industry, at Florence, S. C. Twenty varieties were obtained. These were supplemented by typical "Palma Christi" and a few selections of what appeared to be distinct varieties from the miscellaneous assortment included in the Bombay seed. They were planted in a field surrounded by an abundance of infective material. Results were not as satisfactory as might have been wished, owing to a number of adverse circumstances, but general conclusions can be drawn. Plants of the more ornamental type, with stalk, foliage, and sometimes pods in different shades of red or reddish green, were the most resistant, the pods especially being coarser and obviously tougher. All the smaller, many-branched plants, which by their yield indicated commercial possibilities, showed high susceptibility to the disease. Many of them at the close of the season had as high as 90 per cent of the inflorescences affected, and practically all over 50 per cent. Cross pollination in a castor-bean field probably occurs very extensively. It would require years of work to develop pure strains and then to select and breed for desirable qualities combined with resistance before permanent results in the way of control could be secured.

CASTOR BEAN HULLS FOR FERTILIZER

Castor bean hulls have a high potash content, consequently there was considerable agitation during the winter following the harvesting of the

1918 crop for their use as fertilizer. The one objection to this procedure, in case castor beans had become a staple crop, would have been the widespread distribution of the gray mold that might have followed. This danger might have been overcome by grinding the hulls and mixing them with acid phosphate and other ferilizers at the fertilizer plants. The close contact of the hulls to the strong acid of the fertilizer would possibly have quickly killed the fungus. No investigations to check this up were undertaken, however.

CONTROL EXPERIMENTS

Various plantings of castor beans were made in the spring of 1919 on different areas of vacant ground at Orlando, Fla. The sites were chosen particularly with reference to proximity to infected fields of the previous season's growth. Lot A consisted of an area of about ½ acre cut out in the middle of a large field which had been badly diseased in 1918. The soil was a fertile "hammock-land" type, bordering one of the numerous small lakes in the vicinity of Orlando. It required no fertilization but was prepared by first pulling up and clearing away the remains of last year's plants, then simply plowing and harrowing. This was done the last week in February. The field was divided into 1/10-acre sections for convenience, and plantings were made of various lots of seed during the first week in March. A frost the night of April 2 killed many of the plants which were well up, so a day or two later replantings were made, these being final. Lot B consisted of a portion of a lot 50 by 150 feet, approximately ¼ mile from Lot A, which was the nearest of last year's plantings. Lot C consisted of another piece of ground at a distance of approximately ½ mile from the first field, covering an area 200 by 150 feet in dimensions. This was upon sandy soil and required considerable fertilizer. Plate 2, A, is a general view of this field upon which the greater part of the control experimental work was conducted.

During the summer of 1918, when the disease appeared at its height, the standard fungicide, Bordeaux mixture, was recommended provisionally as a possible means of control (*16*, *17*). This spray has been recommended more or less extensively for that type of trouble, as for instance by Stevens and Hall (*15*) for Botrytis diseases (Botryose) in general. As a rule, however, the only practicable means for controlling Botrytis diseases of crops appears to be the application of sanitary measures, namely, the destruction of diseased parts and of diseased plant débris and thinning or otherwise improving ventilation and doing away with excessively moist conditions. It seemed advisable to give fungicide application a thorough trial for this disease, however, and this was done in lot C during the summer of 1919.

A few preliminary experiments pointed out as a method of procedure the following: The portion of the field to be tested was divided into four sections, each containing nine 150-foot rows. These four sections were to be treated, respectively, with (1) Bordeaux dust, (2) lime and sulphur dust (containing 10 parts sulphur to 90 parts lime), (3) Bordeaux spray, 4-4-50, and (4) lime-sulphur spray, 1-30. The latter was found to be definitely injurious in that strength, so after the first application 1-40 was applied. The first three rows in each section were treated twice a week, on Wednesday and Saturday; the second three once a week, on Saturday; and the third three left untreated as controls. Before starting the experiment the plants were examined thoroughly and all diseased and ripe spikes cut off and dropped to the ground.

The applications were made according to schedule for a period of six weeks, except for slight variations on account of unfavorable weather. The sprays were applied with a knapsack sprayer; the dusts, with a hand-operated dusting machine of the fan blower type, suspended by straps from the shoulder. The applications were very thoroughly made, from both sides, so it is very certain that the spikes were well covered with the fungicides. On Saturday of each week, before the regular treatment was applied, a count was made of all diseased and disease-free spikes. The count included immature spikes that were bursting through the sheath, but none younger than that. In this way an accurate comparison was made of the effectiveness of the different treatments. At the same time all the diseased spikes were cut and allowed to fall to the ground, thus providing rows of mold-free plants for the following week.

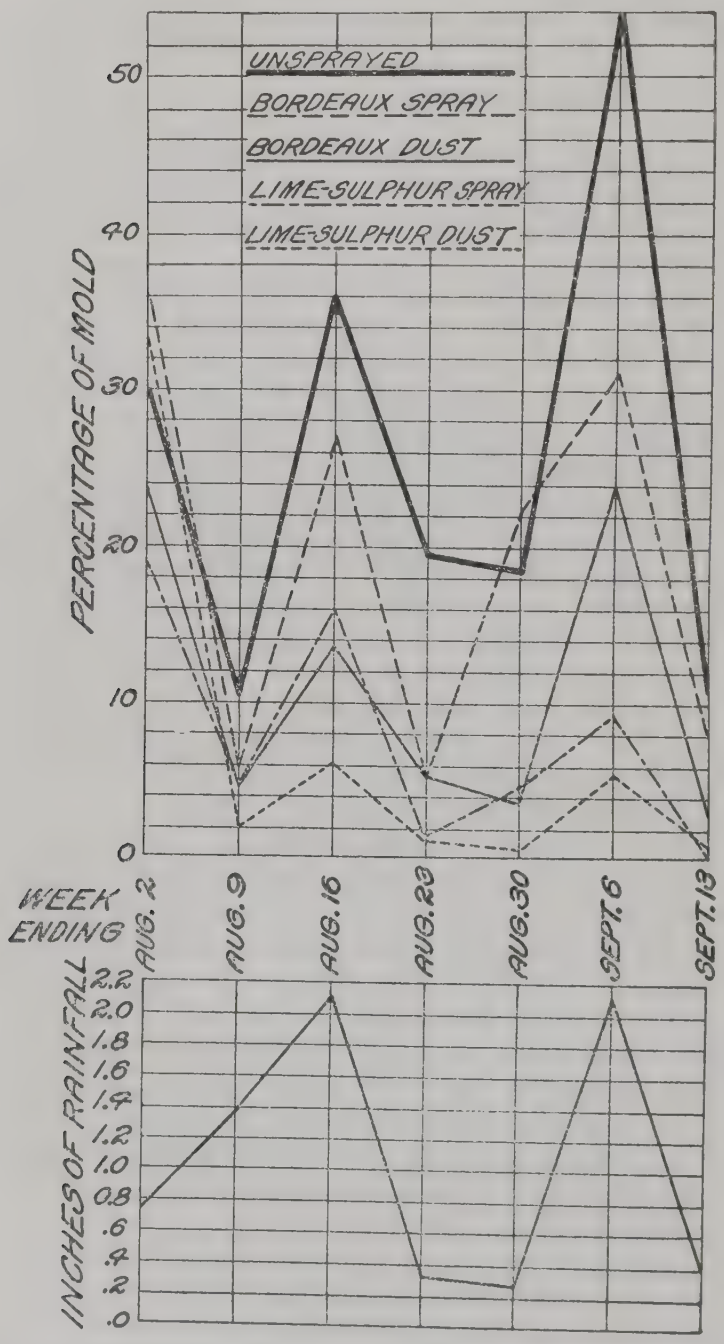

FIG. 4.—Results of spraying experiment from August 2 to September 13, 1919. The graphs show the percentage of mold developing on castor-bean plants, week by week, for each of the fungicides used; and, for the purpose of comparison, the weekly rainfall over the same period.

In connection with the control experiment a test was conducted according to the method described by Winston and Fulton (*24*) to determine the persistence on the plant of the copper preparations used. Briefly, the particular methods used were as follows: At the time the counts were being made each Saturday liberal samples of spikes from the Bordeaux dust and Bordeaux spray-treated plots were collected at random in paper bags. The samples included about an equal number of diseased and disease-free spikes. Out of each lot 200 gm. were weighed out and washed thoroughly in 500 cc. distilled water into which 1 cc. of nitric acid had been poured. Any copper sulphate

present was, of course, promptly dissolved. Test-tube samples of these solutions, in duplicate, were then set up in racks and treated with potassium ferrocyanid. The color intensities produced were compared with those in a graded series of copper-sulphate solutions of known concentration, and readings were taken accordingly from the nearest in color in terms which were readily convertible to milligrams of copper per 100 gm. of the sample. It is evident that each unit of weight of such material as castor-bean inflorescences, containing a large proportion of heavy, immature pods, would have much less exposed surface than the same weight in leaves, as given in the paper referred to (*24*); consequently the results obtained in this case are not comparable with theirs. They are comparable, however, within this experiment, one condition with another.

In order to establish a basis of comparison to determine persistence of the different materials on the plants, a special application was made at the close of the series. The spray was allowed to become thoroughly dry and the dusted spikes to blow in the wind for a couple of hours; then samples were collected and tests made for copper, with results as follows: Three of the dusted samples gave 41.7, 62.6, and 50 mgm. copper per 100 gm. of sample, averaging practically 50 mgm. per 100 gm.; two of the sprayed samples gave identical results—25 mgm. copper per 100 gm. of sample. Consequently these figures are taken as very rough bases upon which to calculate percentages of persistence of the copper applications. The results of the spraying experiment, week by week, are given in Table II in figures representing total number, number of moldy, and percentage of moldy spikes. In addition, figures are given showing the weight in milligrams of copper, as such, per 100 gm. of plant parts sprayed, at the close of each week, together with the relation between that and the "perfect" persistence, expressed in percentages. Some of the results are more graphically presented in Figure 4.

Several interesting facts can be ascertained by study of this table and figure 4. In the first place, there is a very definite correlation between rainfall and the development of mold in all the plats. During a week when less than ½ inch of rain fell, as a rule less than 20 per cent of the flower clusters in the untreated plats developed the mold; when over 1 inch fell, more than 25 per cent; and in one week over 50 per cent became moldy. An exception to this was the week ending August 9, when the rainfall was bunched and several days were dry. The difference in the persistence of the copper applications can also be traced, on the whole, to rainfall, though some inconsistencies appear to be present. These are due, as a rule, to the time of application of the fungicides in relation to some of the heavier downpours of rain. For example, during the week ending September 6, the single application of Bordeaux dust almost entirely disappeared, whereas a comparatively large proportion of the second application, made after most of the rains were over (*20*) remained (fig. 5). The copper of the Bordeaux spray was, on the whole, more persistent than that of the dust. The anomaly evident for the week ending September 6, in which the Bordeaux spray has a sticking quality better than 100 per cent, can be explained only by the supposition that an unusually heavy application was made in the middle of the week, after the rain was over for the week.

The comparative efficiency of the fungicides used is best shown in the graph (fig. 4) in which curves are used to represent the percentages of moldy spikes left at the end of each week, under the different treatments. Only the twice-a-week applications are compared here. In so far as control of the mold is concerned, the sulphur applications appear to be

the much more efficient, with the advantage, between the two, in favor of the lime and sulphur dust. However, a burning and consequent serious injury to the plants was very evident with both sulphur compounds. This injury was sufficient to put these preparations out of consideration as practical remedies. The Bordeaux dust, contrary to what might have been expected from the comparisons of relative persistence, was more efficient than the spray. No injury whatever resulted to the plants from the applications of the copper compounds.

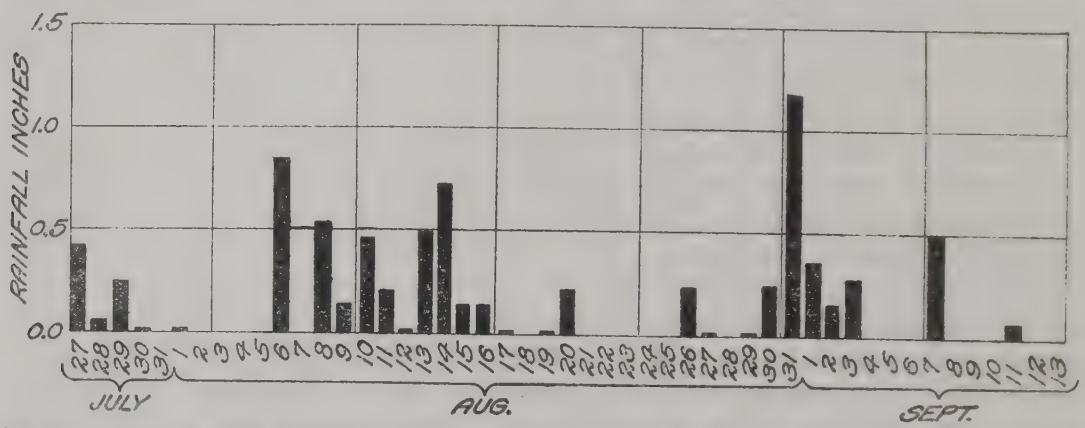

FIG. 5.—Diagram showing the daily precipitation record at Orlando, Fla., over the period covered by the spraying experiment, July 27 to September 13, 1919.

TABLE II.—*Results of applications of fungicides and persistence of copper applications*

Week ending—		Bordeaux dust.			Lime sulphur dust.			Bordeaux spray 4–4–50.			Lime sulphur spray 1–40.		
		Twice.	Once.	Control.	Twice.	Once.	Control.	Twice.	Once.	Control.	Twice.	Once.	Control.
Aug. 2	Total spikes	287	222	208	184	284	272	201	167	110	145	116	46
	Moldy spikes	67	70	61	60	55	68	71	43	37	27	17	14
	Percentage moldy	23.3	31.5	29.3	32.6	19.4	25	35.3	35.7	33.6	18.6	14.7	30.4
	Copper (mgm. per 100 gm. spikes)	5	0	.....	.....	.....	.....	4.2	4.65	.....	.....	.....	.....
	Percentage persistence	10	0	.....	.....	.....	.....	16.8	18.6	.....	.....	.....	.....
9	Total spikes	300	225	206	247	310	262	225	233	171	99	197	82
	Moldy spikes	14	22	16	5	8	19	13	26	19	5	14	13
	Percentage moldy	4.7	9.8	7.8	2	2.6	7.3	5.8	11.2	11.1	5	7.1	15.8
	Copper (mgm. per 100 gm. spikes)	7.8	(2)	.....	(3)	.....	.....	10.45	0	.....	.....	.....	.....
	Percentage persistence	15.4	(2)	.....	.....	.....	.....	41.8	0	.....	.....	.....	.....
16	Total spikes	433	285	311	314	474	450	402	371	235	296	253	112
	Moldy spikes	59	57	129	19	124	134	107	125	83	47	80	40
	Percentage moldy	13.6	20	41.5	6.1	26.2	29.8	26.6	33.7	35.1	15.9	31.6	35.7
	Copper (mgm. per 100 gm. spikes)	6.9	0	.....	(4)	.....	.....	8.3	8.3	.....	.....	.....	.....
	Percentage persistence	13.8	0	.....	.....	.....	.....	33.3	33.3	.....	.....	.....	.....
23	Total spikes	476	335	268	484	324	366	387	339	194	277	124	100
	Moldy spikes	26	50	47	6	23	65	21	48	41	4	10	22
	Percentage moldy	5.5	14.9	17.5	1.2	7.1	17.8	5.4	14.2	21.1	1.4	8.1	22
	Copper (mgm. per 100 gm. spikes)	33.3	4.3	.....	.....	.....	.....	16.7	4.65	.....	(5)	(5)	.....
	Percentage persistence	66.7	8.6	.....	.....	.....	.....	66.7	18.6	.....	.....	.....	.....
30	Total spikes	473	330	278	522	475	366	319	240	193	320	243	91
	Moldy spikes	17	36	34	3	7	51	71	65	36	14	15	26
	Percentage moldy	3.6	10.9	12.2	.6	1.3	13.9	22.3	27.1	18.7	4.4	6.2	28.6
	Copper (mgm. per 100 gm. spikes)	10.4	4.3	.....	.....	.....	.....	17.9	4.3	.....	(4)	(4)	.....
	Percentage persistence	20.8	8.6	.....	.....	.....	.....	71.6	17.2	.....	.....	.....	.....
Sept. 6	Total spikes	528	351	309	399	535	447	488	344	201	319	263	100
	Moldy spikes	126	149	157	22	150	229	151	181	113	30	71	57
	Percentage moldy	23.9	42.5	50.8	5.5	28	51.2	30.9	52.6	56.2	9.4	27	57
	Copper (mgm. per 100 gm. spikes)	33.3	(2)	.....	.....	.....	.....	29.5	4.3	.....	(4)	(4)	.....
	Percentage persistence	66.7	(2)	.....	.....	.....	.....	118	17.2	.....	.....	.....	.....
13	Total spikes	484	335	285	645	571	403	427	309	121	295	222	60
	Moldy spikes	14	24	52	5	38	42	34	32	12	2	4	9
	Percentage moldy	2.9	7.2	18.1	.8	6.7	12	8	11.8	10	.7	1.8	1.5

[1] The spraying was postponed until Monday on account of rain; dusts applied as usual.
[2] Trace. [3] Burning evident. [4] Burned. [5] Much burning.

All the applications used controlled the disease to some extent, as can be seen at a glance. The failure to get anything like complete control was undoubtedly due not so much to the ineffectiveness of the fungicides against the parasitic mold as to the extreme rapidity with which the plants grew and exposed unprotected surfaces to infection by the myriads of spores that were present at all times. New buds would unfold overnight. Young inflorescences would double in size in 48 hours. Male blossoms would open up, uncovering the anthers, thus multiplying the exposed surface many fold and in addition furnishing ideal conditions for infection. Young pods themselves would rapidly increase in size and expose unprotected surfaces. It is evident, therefore, that spraying is out of the question in so far as practical control of the disease is concerned. Three or four sprayings during the season would be impracticable in so far as expense is concerned, to say nothing of the two or more applications per week that would be necessary to produce results.

SEED-TREATMENT

It has been shown that this destructive disease was carried into America by means of the seed and that the seed is perhaps its most important carrier from one section to another. A seed treatment that would destroy the fungus without injuring the seed would therefore be a step toward prevention of the disease. In a seed-treatment experiment carried out in March, 1918, at Orlando, Fla., lots of 200 seeds each were given the following treatments: 1 to 240 formaldehyde (commercial 40 per cent formalin) for 5, 10, 15, 20, 30, 45, and 60 minutes, and murcuric chlorid 1 to 1,000 for equal periods. After the treatments, the seed were planted in rows, under natural conditions. A month later a careful count was made of the germination and growth in each row. The results are given in Table III.

TABLE III.—*Germination of seeds treated with formaldehyde and mercuric chlorid*

Period of treatment (minutes).	Percentage germination.	
	Formaldehyde.	Mercuric chlorid.
5	68.5	73
10	53.5	57.5
15	63	55
20	55	67
30	60.5	63.5
45	64	74
60	67.5	71

A frost on April 1 undoubtedly killed some of the plants, but the results are roughly comparable. For either treatment, germination was practically as high for 60 minutes' immersion as for 5. It is therefore certain that no injury is likely to be done the seed by any reasonable chemical treatment.

Seeds formed in an inflorescence that has been attacked by the gray mold are lighter in weight than normal healthy seed; in fact the worst of them are so light as to be blown out by the blower in a seed-cleaning machine. The castor beans shown in Plate 7, E, are examples, light in weight and worthless as seed. The living fungus inside the tough seed

coat may lie dormant for months. It is very unlikely that any of the heavier seed carry infection inside the seed coat. Simply floating off the lighter seed in water, therefore, would eliminate a large part of the danger of transportation of the disease through this means. Experiments in this connection have shown, however, that a considerable proportion of the apparently normal seed will float in water when first immersed, and also that seed which were heavy enough to sink at first may rise later. In a specific trial, half of a lot of 50 seeds floated when first put in the water; in 30 minutes 8 more had risen; after an hour longer 8 were floating and 42 were on the bottom. Several different lots acted the same way, in general. It is recommended, therefore, that, in order to combine the floating process with seed treatment, some such precedure as the following be practiced, if there is chance that the seeds are infected. The seeds should be immersed in water in a large receptacle and left for about 1½ hours with frequent stirring. Then the disinfectant, preferably formaldehyde, should be added in the proper proportion, and the seeds left for 15 minutes longer. At this time all those that are floating should be skimmed off, together with all trash, and discarded in so far as use for seed is concerned. It is true that many good seeds will probably be discarded along with the poor, but they will still be available for oil, and the certainty will be the greater that only the best and cleanest seed is reserved for planting.

GENERAL RECOMMENDATIONS FOR CONTROL

Regardless of the freedom of seed from disease, planting castor beans in a locality where the disease is present is almost certain to bring about a diseased crop. Observations made in April, 1921, three years after the war emergency plantings of 1918, showed the disease to be still present and active at Orlando, Fla. Several castor bean plants that had survived the mild winter of 1920–21 and had grown to be trees 15 feet high and 6 inches in diameter at the base were covered with fruiting spikes in different stages of maturity. About 50 per cent of these were affected with the typical gray mold. The first requirement, therefore, toward the growing of a mold-free crop would be planting in a mold-free locality. Planting in locations where climatic conditions are not favorable to the disease would be a still better safeguard, in that no infections could occur from chance introductions of the disease on seed or otherwise. The map (fig. 1) shows the localities in America where the disease occurred and where it did not occur, in 1918. Several States, in some of which in fact, the best yields were produced, are seen to be suitable in this respect. In case the necessity should ever recur for heavy plantings in America, this should be one of the primary considerations. A study of the rainfall maps over a considerable period might be advisable in order to be certain that the 1918 conditions were not exceptional. After choosing the localities where the planting is to be done, the source of seed should be considered. If there is any doubt about the cleanness of seed, 15 minutes' treatment, in either formaldehyde solution or mercuric chlorid, as recommended above, should be given as an additional safeguard. Only as a last resort, to prevent general spread from a limited primary infection, can spraying or dusting be recommended. If accompanied by complete destruction of all the disease that has been found, and if repeatedly and frequently applied, a fungicide might be helpful. But on the whole such measures are not recommended as

practicable. Control measures, therefore, are to be wholly preventive rather than to any extent destructive of the disease after it once gets a start.

SUMMARY

(1) Castor beans were cultivated on a large scale in 1918, as a war emergency measure. A number of insect pests and diseases appeared, the most serious of which was a blight of the inflorescence caused by a new species of Botrytis, the perfect stage of which was later found and described (*Sclerotinia ricini* Godfrey).

(2) The disease was found throughout the State of Florida, and also in Mississippi, Louisiana, and Texas. It was not to be found in fields in South Carolina, Alabama, Georgia, Tennessee, or Arkansas. From 10 per cent up to 100 per cent loss was sustained by growers in the affected districts.

(3) A succession of several continuously wet days was requisite to the development and spread of the pathogene. In localities where there was much of this kind of weather during the growing season the percentage of loss was high; where there was less, the loss was correspondingly lower; where summer rains were of brief duration and followed by long periods of dry weather the disease did not occur.

(4) The disease is a typical Botrytis blight. The inflorescences or "spikes" are attacked, in all stages of development, with heavy growth of mold and complete destruction. The leaves and stems are also attacked occasionally. During the winter and spring following, sclerotia appeared in abundance, at first on the old spikes and later on the stalks of the plants.

(5) The causal organism is very readily isolated, since it is a rapid grower on most of the common culture media and outgrows most contaminating organisms. About 50 isolations were made from different sources, mostly from single conidia. Its appearance in culture is that of a typical Botrytis, with the usual abundance of gray mold and black sclerotia. It is characterized microscopically by its comparatively small globose spores in dense heads and the constant dichotomous divisions of the conidiophores. Appressoria are mostly microscopic. Microconidia occur.

(6) Attempts made to induce a perfect stage to develop during the winter of 1918–19 were at first not successful. Early in March a limited number of cultures showed apothecial stalks arising from sclerotia. Single ascospore strains were isolated from apothecia which matured, and the connection with the Botrytis stage was established. Later a great abundance of apothecia were produced under artificial conditions and were found in nature.

(7) The fungus is homothallic. Freezing, drying, or an extended rest period are not requisite to the development of apothecia from sclerotia. Factors necessary before this will happen are reasonably moist conditions, a temperature near the optimum for vegetative growth of the fungus, a reasonable amount of air space, and light.

(8) The perfect stage is a true Sclerotinia, smaller than the more common forms. Ascospores are discharged forcibly in a visible cloud when the apothecia are subjected to a sudden change from moist to dryer conditions. Frequently all eight spores cling together. Ascospores may germinate directly, with the production of a germ tube and hyphæ indistinguishable from those of the Botrytis stage. Microconidia have been observed on germinating ascospores.

(9) A complete technical description of the fungus is given.

(10) It is proved that the pathogene was brought to America from India with the seed.

(11) Numerous inoculations, reisolations, and reinoculations were made with the use of single conidium and single ascospore strains of the fungus. Most of the inoculations were made by the application of conidia, with the use of special waxed paper moist chambers. In addition infection was secured by the application of ascospores alone.

(12) Histological studies showed that penetration of the cuticle, apparently by mechanical means, was necessary before any injury to the host occurred. After penetration disorganization of the host tissue was rapid and complete. The fungus was found within the caruncle and even beneath the seed coat of seed that had been attacked before maturity.

(13) The fungus appears to be practically limited to the castor bean as a host plant. Of a large list of other plants exposed to infection, only three other plants of the family Euphorbiaceæ and a species of Pelargonium were slightly infected, and infection did not develop further under the conditions that favored rapid spread on Ricinus.

(14) Some difference in varietal susceptibility was noted, the coarse ornamentals in general being more resistant than the commercial seed-producing sorts. Nothing promising in the way of control by this means was evident.

(15) Extensive experiments indicated that control by the application of fungicides was entirely impracticable once the disease was well established.

(16) Castor bean seed are not readily injured by any reasonable chemical treatment. A combination of a floating process, to remove all light-weight seed, with a formaldehyde treatment, would seem to be a practicable means of preventing the introduction of the disease from one locality to another by means of the seed.

(17) Control of this disease in the event of any future heavy planting should consist primarily of prevention. The use of clean seed combined with a judicious choice of districts to be planted, based primarily upon freedon from recent occurrence of the disease, should result in the production of a mold-free crop.

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PLATE 1

A.—Young castor bean inflorescence recently infected with *Sclerotinia ricini*. Note the center of infection with heavy production of gray mold, the very recently infected spots exuding amber-colored drops of liquid, and the variation in stage of development of the flowers, both male and female.

B.—Portion of the stalk of a castor bean plant with sclerotia, developed in the subcortical layers, appearing at the surface.

C.—Axis of an old diseased inflorescence, with the pods long since fallen. Note the sclerotia developing at the base.

D.—Apothecia and sclerotia of *S. ricini*.

Reproduced from painting by J. Marion Shull.

A
B
C
D

A
B

PLATE 2[1]

A.—Portion of an experimental castor bean field at Orlando, Fla., upon which observations were made and experimental sprayings conducted in 1919. Note in addition the cloud effects which are typical of the summer season in most parts of Florida. A shower has just occurred in the distance from clouds shown in the upper left-hand corner of the picture. A storm is rising in the other direction, as evidenced by the large dark cloud shown. Such storms pass over quickly and are followed immediately by sunshine, but their frequency, combined with the relatively high humidity, is conducive to the rapid spread of the gray mold.

B.—A single Bombay type castor bean plant, the majority of whose spikes are affected with the gray mold. De Land, Fla., August 22, 1918.

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[1] All photographs used in illustrating this paper were taken by the author except where otherwise indicated.

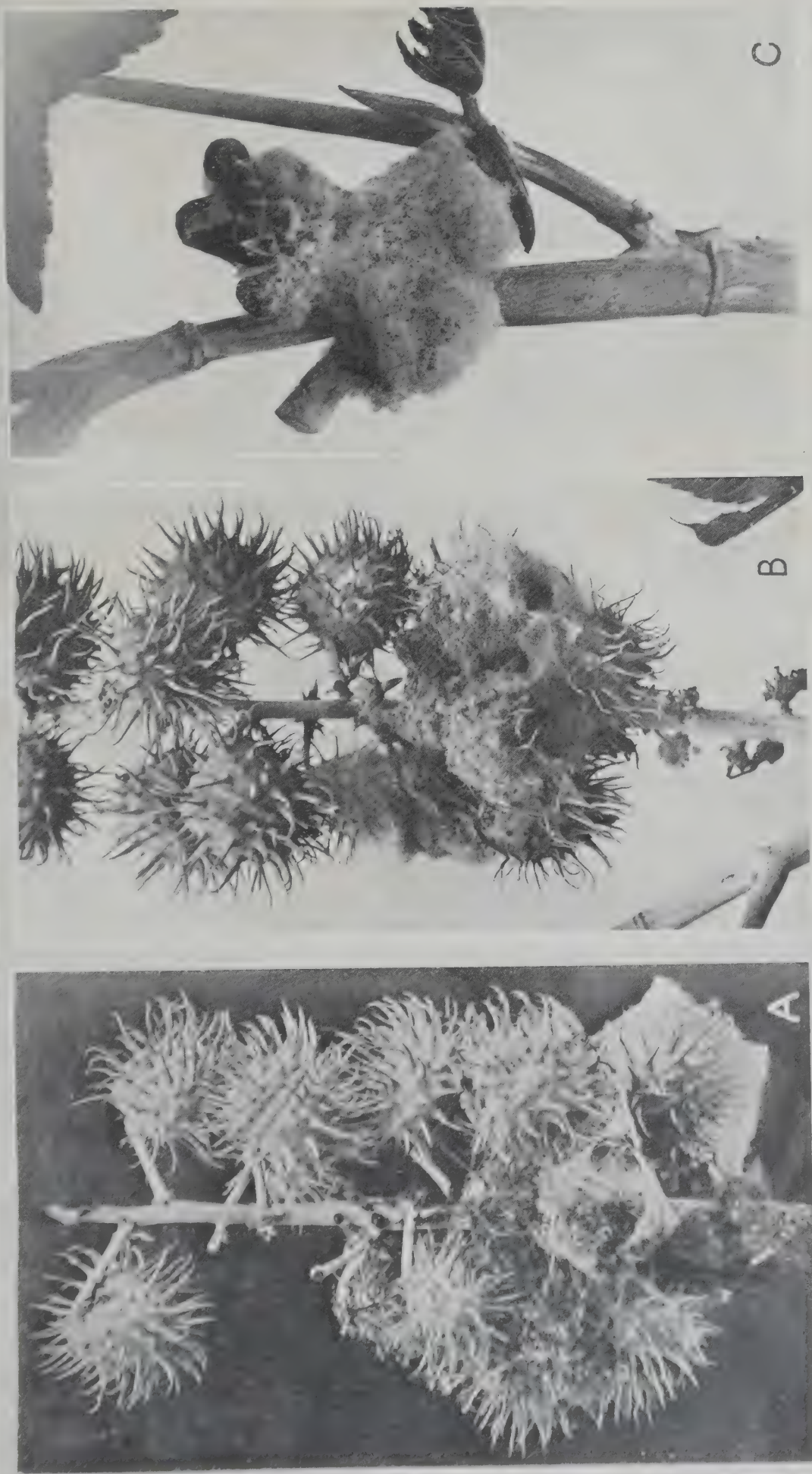
PLATE 3

Castor bean inflorescences infected with *Sclerotinia ricini*.

A.—A nearly mature spike, showing typical appearance of the mold under natural conditions. Note drops exuding from new infections on the axis. × 3/4.

B.—A similar spike with heavy production of mold. × 3/4.

C.—A young spike infected by inoculation with ascospores. The mold was developed, after infection occurred, by holding the specimen for 38 hours under a bell jar. Orlando, Fla., May, 1919. Natural size.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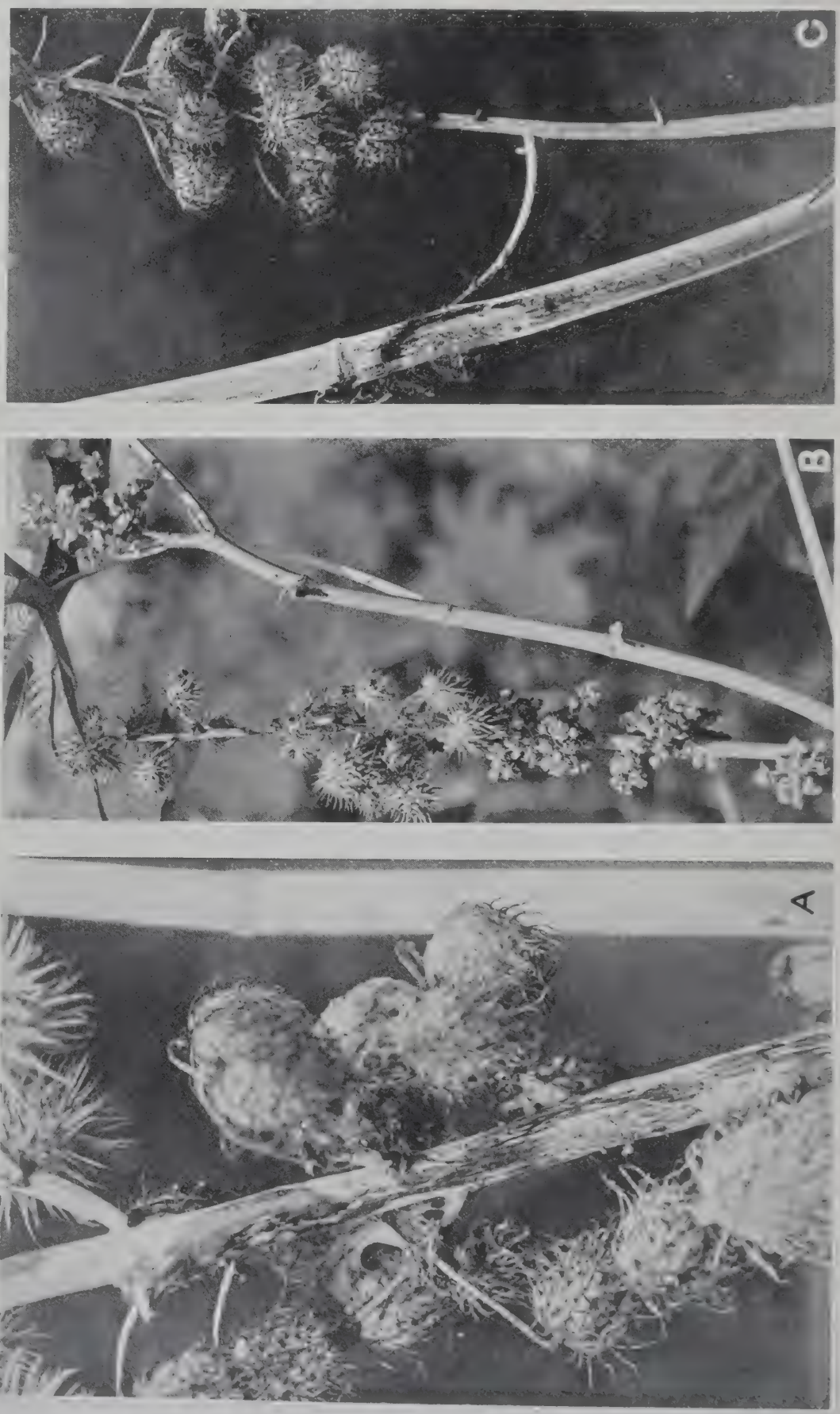
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PLATE 4

A.—Axis of a nearly mature spike almost completely girdled by the fungus. The pods in the center of picture look almost the same as mature pods, but the seeds in them are light and worthless. De Land, Fla., August, 1918. Natural size.

B.—A well-developed spike doomed to destruction, the axis being completely girdled. Note the young spike, also infected. De Land, Fla., August, 1918. × 1/3.

C.—A canker on a stalk, produced by the gray mold. The spike shown was infected when still green and contains worthless light-weight beans. De Land, Fla., August, 1918. × 1/2.

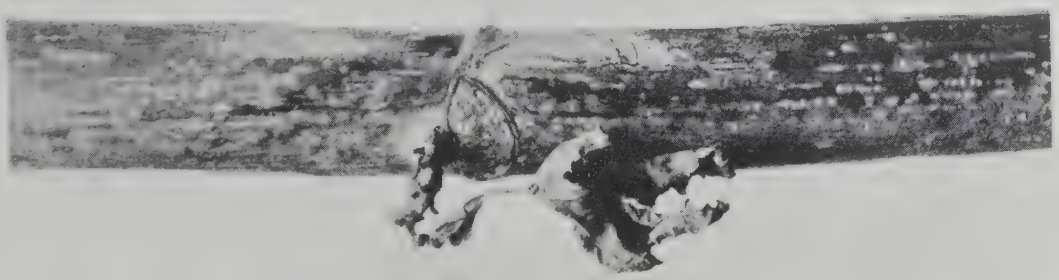
PLATE 5

Winter stage of *Sclerotinia ricini*, Orlando, Fla., February, 1919.

A.—Bases of old diseased inflorescences, showing sclerotia developed in the early spring. × 3/4.

B.—Canker developed on the side of a castor bean stalk from a diseased spur. Note early stage of sclerotium development. × 3/4.

C.—A weak spring growth on the side of an old castor bean stalk. It has become infected with the gray mold and has carried infection into the stalk, producing a canker. × 3/4.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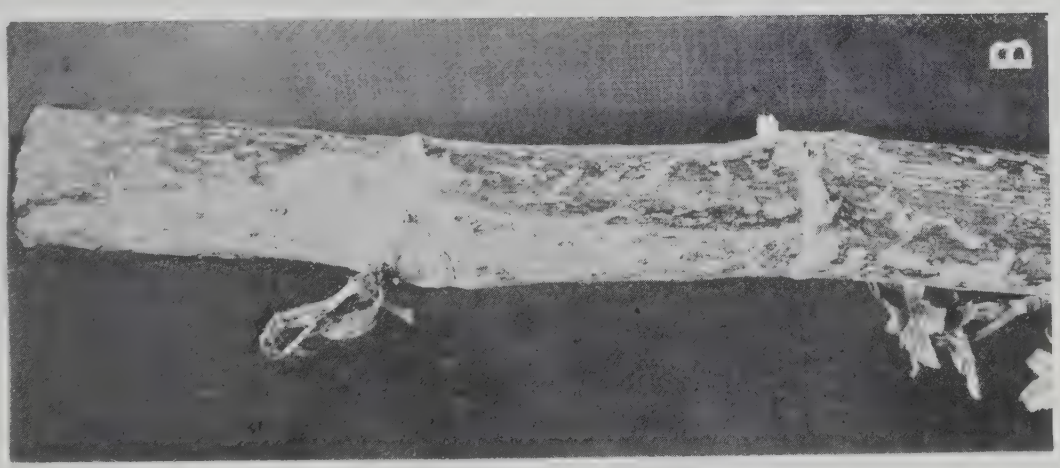
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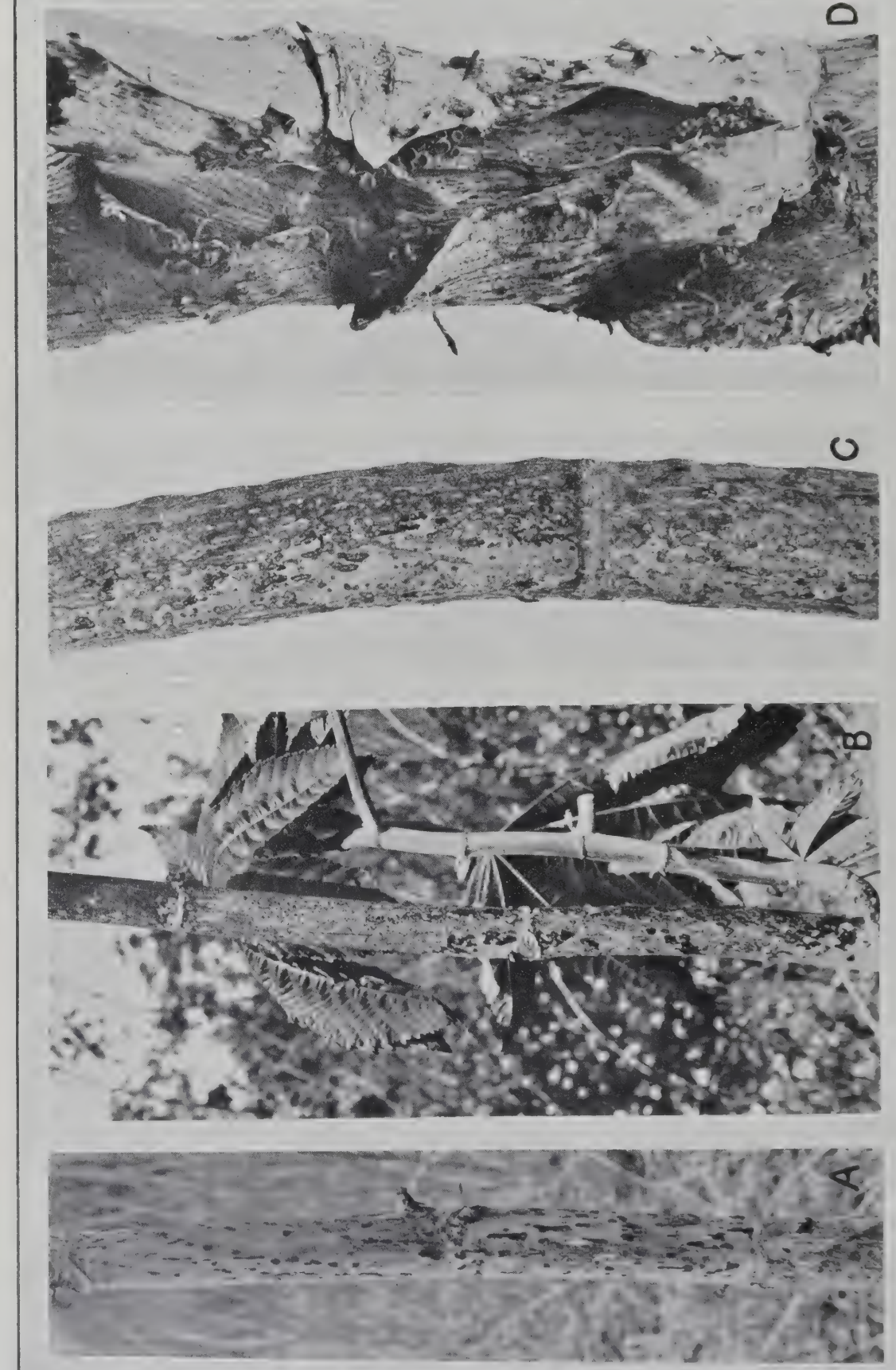
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PLATE 6

Sclerotinia ricini on castor bean stalks, Orlando, Fla.

A.—Sclerotia developed on a stalk, following infection through a spur. May, 1919. × 1/3.

B.—Sclerotia on the surface of a stalk. Note the vigorous, healthy growth developing from below the diseased region. May, 1919. × 1/3.

C.—Superficial and subcortical sclerotia. Note the humps produced by the latter. May, 1919. × 2/3.

D.—An abundance of apothecia developed under natural conditions on sclerotia still attached to the dead stump of the host plant. July, 1919. × 3/4.

PLATE 7

A.—Full-sized but immature castor bean, with part of the hull torn away. Note the large fleshy white caruncle. × 3.

B.—Similar view of a castor bean and hull taken from a diseased inflorescence. Note the gray mold growing on the pod, seed coat, and caruncle. Orlando, Fla., August, 1919. × 3.

C.—Four castor beans from a diseased spike, showing gray mold growing from the seed coat and caruncle. Magnification indicated by the scale.

D.—Gray mold growing from two castor beans six months after they were taken from the hulling plant at Plant City, Fla. The upper one has the mold growing directly on the bean; the lower one shows the mold grown from the seed to the nutrient medium, where it is rapidly developing.

E.—Seed taken from a diseased inflorescence collected at Alvin, Tex., October, 1918. Note the sclerotia on seed coats and hulls. These beans consist of nothing but shells. Natural size.

A

B

RIC 1 SYSTEM 2
C

D

E

A

B

C

PLATE 8

A.—Photograph showing the inoculation method largely used in the experimental work at Orlando, Fla. A sheet of waxed paper is wound about the spike, inclosing a moist cotton plug to maintain high humidity.

B.—Portion of an inoculated castor bean seedling, showing lesions on the stem and attachment of the cotyledons, one of which has already fallen, and the killed growing point. Photographed by Mr. W. R. Fisher, of the Department of Plant Pathology, Cornell University. × 7/8.

C.—Geranium infected by artificial inoculation with the castor bean gray mold. Note the affected leaf margins, the stem lesion, and the killed growing points. Photograph by Mr. W. R. Fisher. × 3/4.

PLATE 9

Photomicrographs of late stages of infection of male blossom buds by germinating conidia.

A.—Deep indentation produced by an appressorium. Infection has already occurred elsewhere in the bud and host tissues are disorganized. Section rather thick, about 25 μ. Note clinging together of the hyphae, indicating a tangled weft, capable of withstanding considerable pressure.

B.—Another view of tangled hyphae, and indentation beneath an appressorium.

C.—Hyphae of the fungus within the disorganized host tissue. The large, round bodies below are partly developed anthers.

Photomicrographed by the photographic laboratory, United States Department of Agriculture.

B

C

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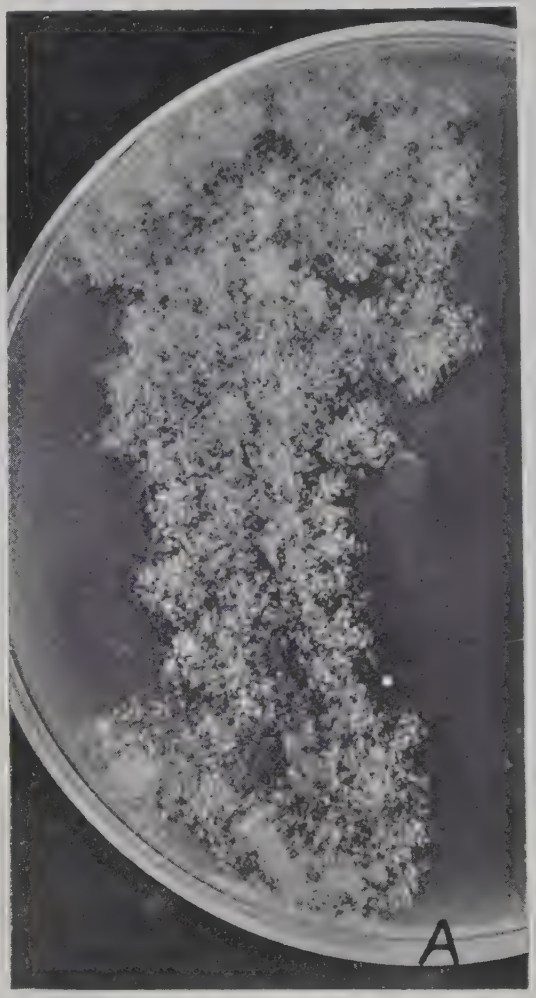
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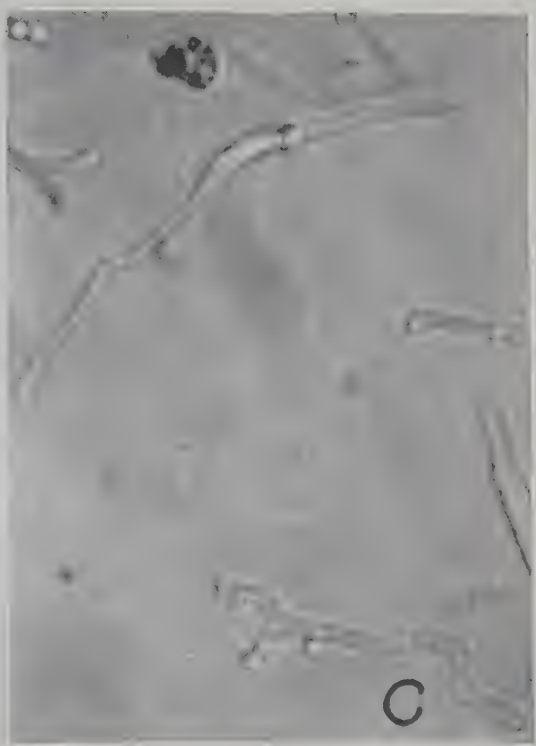
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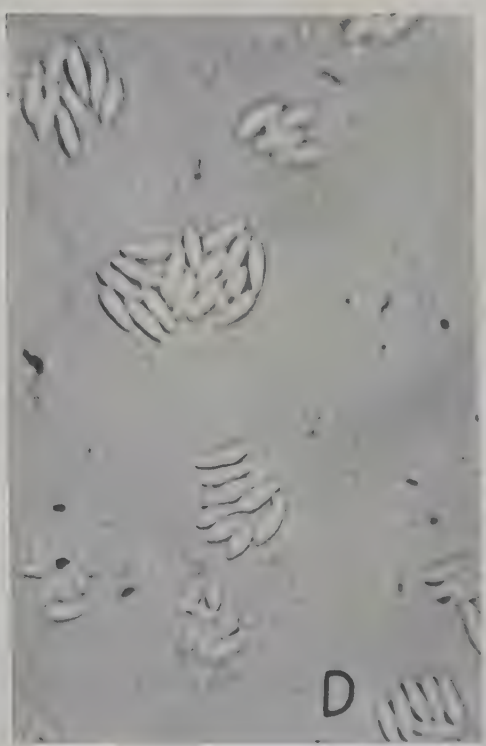
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PLATE 10

Photographs and photomicrographs of *Sclerotinia ricini*.

A.—Gray mold in pure culture on a poured plate of corn meal agar. Culture about 3 days old. Natural size.

B.—Same as A. Approximately × 3.

C.—Germinating ascospores, in some cases with the production of microconidia.

D.—Ascospores as ejected from apothecia in groups of eight.

Photographed by the photographic laboratory, United States Department of Agriculture.

PLATE 11

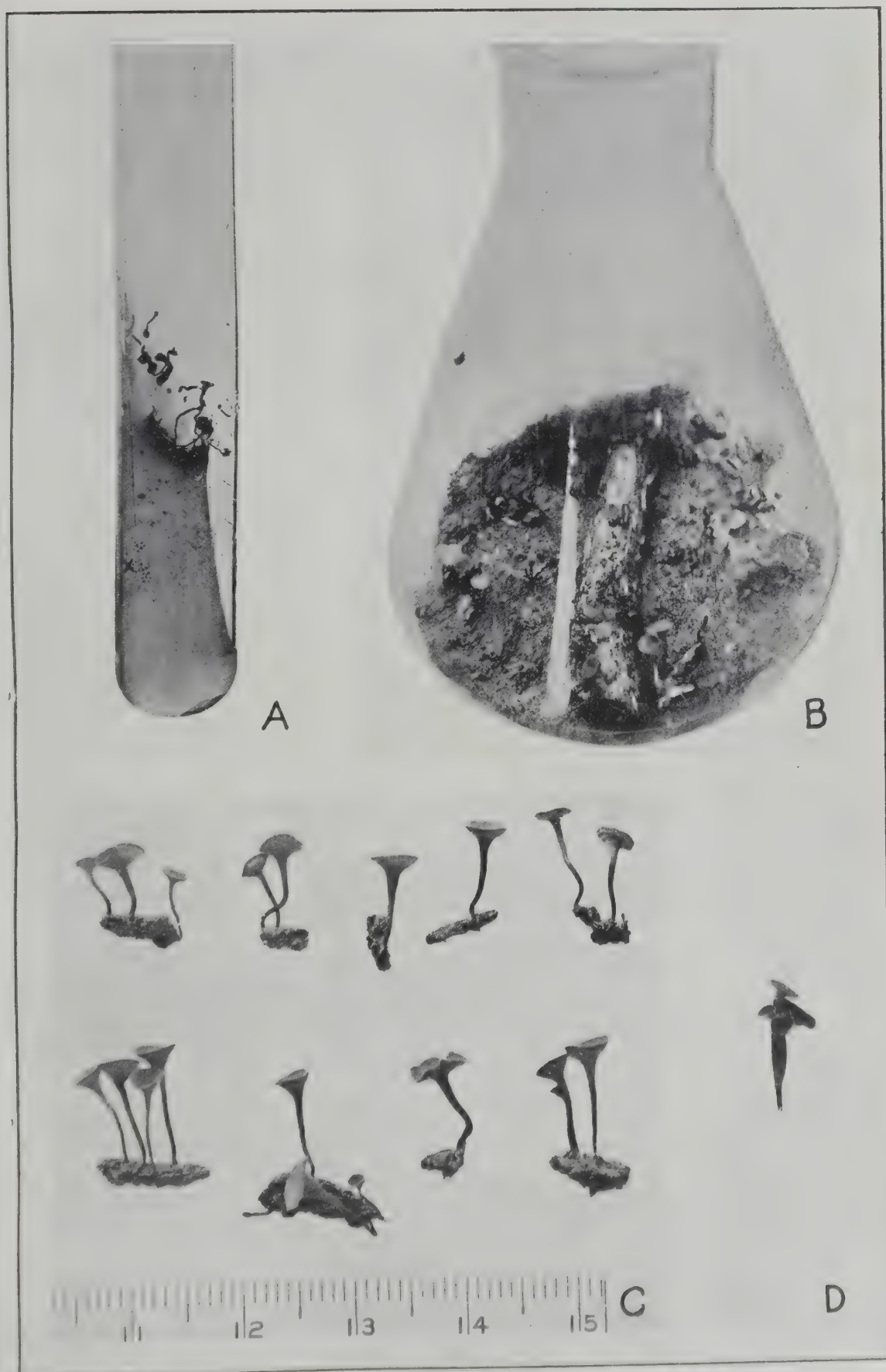
Apothecial stage of *Sclerotinia ricini*.

A.—The first tube in which apothecia developed and reached maturity. The large apothecium in the foreground was the third to mature, and the one from which the first single ascospore isolations were made. Note also the appressoria at *x*. Orlando, Fla., March, 1919. Approximately natural size.

B.—An abundance of apothecia produced from sclerotia placed on moist sand in an Erlenmeyer flask. Orlando, Fla., May, 1919. Approximately natural size.

C.—Apothecia developed as in B, enlarged as indicated, by the metric scale.

D.—An abnormal apothecium with several disks. Somewhat enlarged. Photographed by photographic laboratory, United States Department of Agriculture.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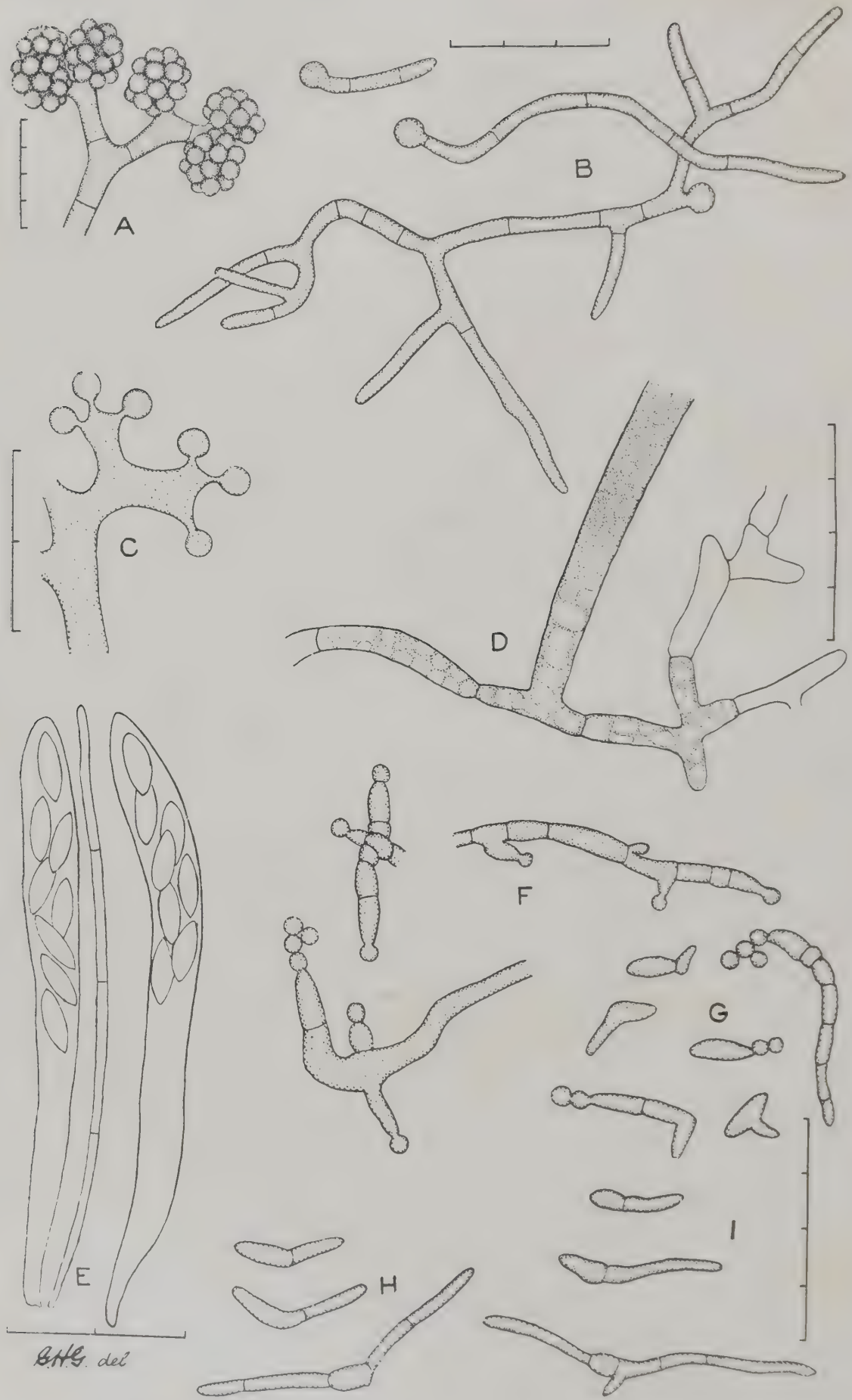
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PLATE 12

Stages in the development of *Sclerotinia ricini*.

A.—Portion of mature head of Botrytis stage. Note dichotomous branching.

B.—Germinating conidia.

C.—Immature conidia, showing attachment to conidiophore.

D.—Base of a conidiophore, hyaline and vacuolate, gradually becoming olivaceous above the substratum.

E.—Asci, ascospores, and paraphysis.

F.—Microconidium production on vegetative hyphae.

G.—The same on germinating ascospores.

H, I.—Stages in germination of two of the ascospores which gave two of the original single ascospore cultures.

Drawings made with the camera lucida. Magnification is indicated by the scales, one space of which, in each case, represents 10 μ.

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PLATE 13

Penetration of a leaf of the castor bean by germinating conidia (except I and J).

A.—Penetration of cuticle by means of a very fine outgrowth almost directly from the spore.

B.—Similar stage of penetration by tip of a germ tube. Swelling of the cuticle seems to be evident.

C.—Two cases of penetration into the cuticle.

D.—A group of germinating conidia. In two places the cuticle has been entered but not completely penetrated. No injury was evident in the epidermal cells.

E.—Another case of partial penetration.

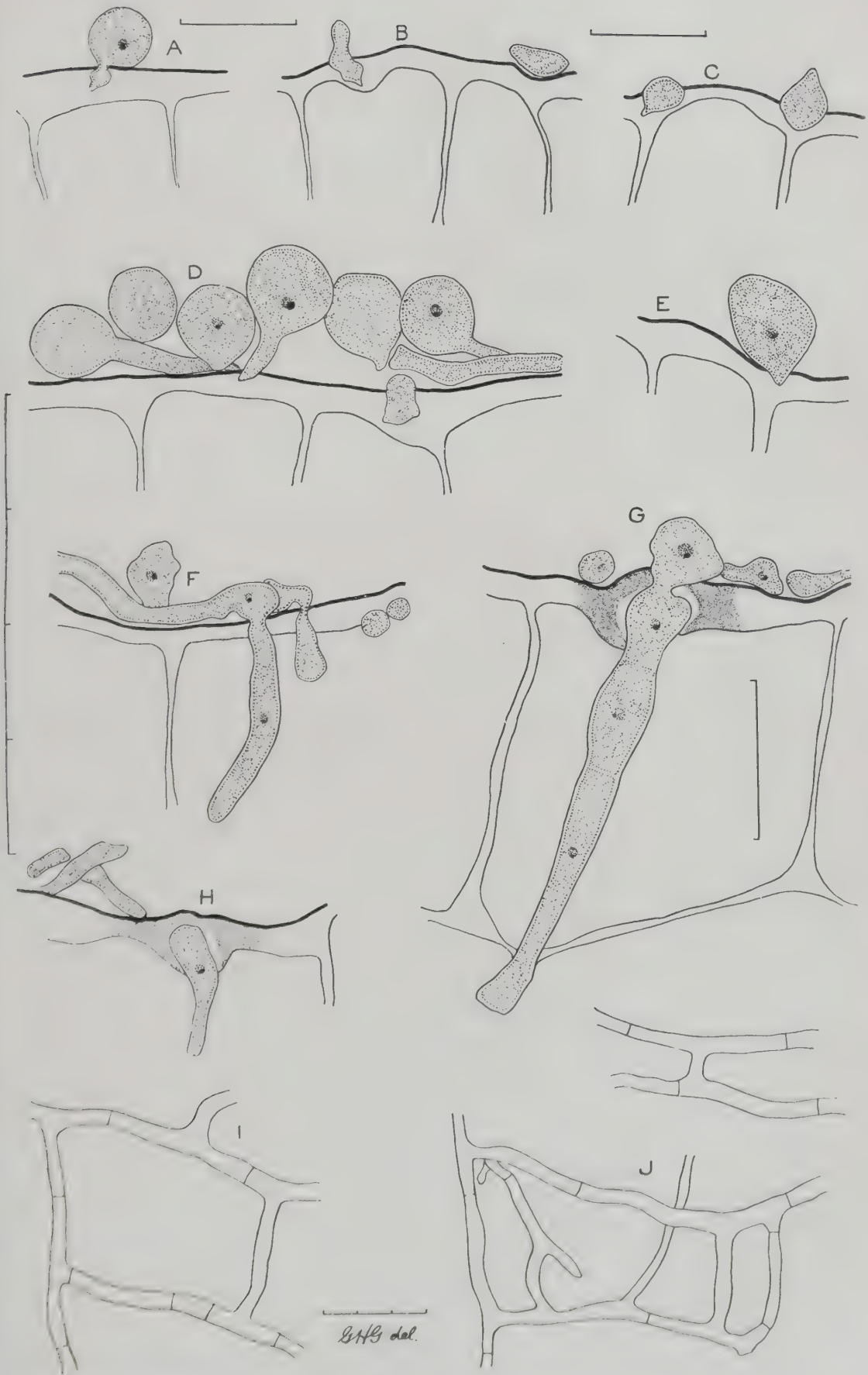
F.—Penetration accomplished and hyphae growing rapidly into the host cells.

G.—Penetration accomplished; the hypha has advanced into the subepidermal layer of host cells. Note the swollen cuticle and the clear region about the penetrating hypha, indicating that some dissolving action has taken place.

H.—Lower part of hypha that has penetrated the cuticle. Apparent dissolving action also seen here.

I, J.—Ansastomosis, or growing together of hyphae in a young culture on clear corn meal agar.

The material was fixed 24 hours after inoculation, some of it showing only very slight injury. Magnification is indicated by the scales, each space of which represents 10 μ.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INFLUENCE OF THE SPECIFIC GRAVITY OF HENS' EGGS ON FERTILITY, HATCHING POWER, AND GROWTH OF CHICKS [1]

By F. E. MUSSEHL, *Professor of Poultry Husbandry,* and D. L. HALBERSLEBEN, *Student Assistant, Nebraska Agricultural Experiment Station*

Several interesting theories have been advanced as to the influence which the specific gravity of the hens' egg may have on its fertility, hatching power, and the subsequent growth of chicks hatched. Devices for the purpose of determining specific gravity of eggs are being distributed, claims being made that the hatching power of eggs is directly related to their specific gravity.

Experimental work for the purpose of studying the principles involved rather than for the purpose of investigating any particular device, were conducted at this station with the results as hereinafter stated.

PLAN OF THE EXPERIMENT

The eggs used for this experiment were produced by hens in pedigree breeding pens, use of the trap nest making it possible to watch unusual variations in size, specific gravity, and other characters. The following data were obtained on each of the eggs used in this experiment: Number of the hen laying the egg, weight of the egg to the nearest gram, specific gravity, record of fertility, death in shell if this occurred, and weight of chick if the egg hatched. On the second group of chicks hatched, data were also obtained on the weight of the chicks at 7 days, 21 days, and 35 days of age.

The specific gravity of all eggs was obtained by using a device which is sold commercially for this purpose. Actual specific gravity values were determined for each of the divisions on the graduated standard of this device and for the various weights of eggs. Translations were made so that our comparisons and groupings are made on a basis of actual specific gravity.

Each of the 958 eggs used in this experiment was given a distinct number, and all chicks hatched were pedigreed separately. All eggs were hatched in the same kind of incubators, the same operator being responsible for all hatches. The chicks were brooded indoors for the first week and were then moved to a colony house heated with a coal-burning brooder stove.

RELATION OF SPECIFIC GRAVITY TO EVIDENT FERTILITY

The summary of our data as obtained from a study of 958 eggs indicates that there is no correlation between the specific gravity of the egg and evident fertility during incubation. Table I consists of a summary of these data.

[1] Accepted for publication Feb. 15, 1922.

TABLE I.—*Relation of specific gravity to fertility*

Specific gravity.	Number infertile.	Number fertile.	Total.
1.01	2	.....	2
1.02	1	2	3
1.03	.....	3	3
1.04	2	24	26
1.05	12	78	90
1.06	35	178	213
1.07	49	264	313
1.08	40	190	230
1.09	12	56	68
1.10	3	7	10
Total	156	802	958

$r = -0.038 \pm 0.022$.

Since there is no apparent correlation between specific gravity and fertility, we have eliminated the infertile eggs in organizing data for the next subject of inquiry, which was the relation of specific gravity to hatchability of fertile eggs. Table II summarizes the results of this study.

TABLE II.—*Relation of specific gravity to hatchability of fertile eggs*

Specific gravity.	Number fertile eggs not hatching.	Number fertile eggs hatched.	Total.
1.01	.....	.....	.....
1.02	2	.....	2
1.03	2	1	3
1.04	19	5	24
1.05	61	17	78
1.06	112	66	178
1.07	142	122	264
1.08	84	106	190
1.09	24	32	56
1.10	5	2	7
Total	451	351	802

$r = 0.204 \pm 0.023$.

The observations summarized in Table II, made on 802 eggs, with a positive correlation of 0.204, indicates a slight relation between the two factors being studied, but the degree of correlation is so slight that it lacks practical usefulness for the poultry producer.

The relation of the specific gravity of the eggs to the probability of the chick's living through the first five weeks, the critical period, was also studied with the chicks hatched in the second division of the experiment.

TABLE III.—*Relation of specific gravity to viability of chicks hatched*

Specific gravity.	Number dying.	Number living.	Total.
1.03	.....	1	1
1.04	.....	.....	.....
1.05	.....	3	3
1.06	2	15	17
1.07	6	46	52
1.08	8	38	46
1.09	5	23	28
1.10	.....	3	3
Total	21	129	150

$r = -0.074 \pm 0.055$.

It has furthermore been stated in commercial literature that chicks hatched from eggs of high specific gravity grow much more rapidly than those hatched from low specific gravity eggs, by virtue of more complete nutritional opportunities in the early life of the high specific gravity chicks. The growth history of 129 chicks were studied with this subject for inquiry, the results being summarized in Table IV.

TABLE IV.—*Relation of specific gravity of eggs to early growth of chicks hatched*

[Expressed in percentage of increase in weight at 35 days over weight at hatch]

Specific gravity.	101 to 200	201 to 300	301 to 400	401 to 500	501 to 600	601 to 700	701 to 800	Total.
1.03	.....	.....	1	.....	.....	.....	.....	1
1.04	.....	.....	.....	.....	.....	.....	.....	.....
1.05	.....	.....	.....	.....	1	1	1	3
1.06	.....	.....	.....	5	4	5	1	15
1.07	.....	2	9	18	11	5	1	46
1.08	.....	.....	2	17	12	6	1	38
1.09	1	.....	3	10	5	4	.....	23
1.10	.....	.....	1	.....	2	.....	.....	3
Total	1	2	16	50	35	21	4	129

$r = -0.113 \pm 0.059$.

This observation made on 129 chicks indicates that there is no correlation between the specific gravity of eggs and the early growth of the chick hatched.

FACTORS INFLUENCING THE SPECIFIC GRAVITY OF EGGS

Willard and Shaw[2] report the analysis of large numbers of hens' eggs, giving the following chemical composition: Water, 65.8 per cent; ether extract, 10.5 per cent; protein, 12.8 per cent; ash in yolk, 1.5 per cent; ash in shell, 9.9 per cent. The specific gravity of the shells of a number of eggs was determined by one of us (Halbersleben) and was found to average 2.14. The average specific gravity of the whole eggs in this

[2] WILLARD, J. R., and SHAW, R. H. ANALYSES OF EGGS. Kans. Agr. Exp. Sta. Bul. 159, p. 143–177, 1909.

group was 1.07, one-half the specific gravity of the shell. The albumen, constituting approximately 10 per cent of the total egg volume, has a specific gravity of 1.31, which is not enough greater than that of the whole egg to have much influence should the protein content of the egg vary as much as one-half of 1 per cent, which is the maximum variation reported from analyses of a large number of eggs as reported by Willard and Shaw.[3] The fat, which constitutes approximately 12 per cent of the total volume of the egg, has a specific gravity of 0.881 (at 100° C.).[4] Slight variations in the fat content of the egg would not greatly influence the specific gravity, and the effect of an increased fat content would be a lower specific gravity.

Variations in shell weight and thickness are therefore most likely to influence the specific gravity of the entire egg, since the specific gravity of the shell is nearly twice as great as that of the albumen and more than twice that of the fat in the egg. That the shell material has a most essential function in the nutrition of the embryo in the egg is known, for the chick at hatching time has five times as much calcium in its skeletal structure as is present in the egg contents. Harcourt and Fulmer [5] report (an average of 37 analysis) 0.03615 gm. of calcium oxid in egg contents, while the analysis of 65 chicks on the twentieth day of the hatch showed a calcium oxid content of 0.19487 gm. Normal eggshells, however, contain at least 2.5 gm. of calcium oxid, so that even thin-shelled eggs contain enough calcium to satisfy all requirements, provided other factors influencing its assimilation are satisfactory.

SUMMARY

Our conclusions based on a close observation of 958 eggs and the subsequent growth of one lot of 150 chicks hatched are as follows:

(1) There is little correlation between specific gravity, fertility, and hatchability of hens' eggs.

(2) The specific gravity of the egg has no relation to the viability of the chick hatched or to its growth rate the first five weeks after hatching.

(3) Variations in the thickness of the shell are more likely to influence the specific gravity of eggs than are variations in protein or fat content.

[3] WILLARD, J. R., and SHAW, R. H. OP. CIT.

[4] SPAETH, EDUARD. DER NACHWEIS DES EIGELBES (EIDOTTERS) IN MEHLFABRIKATEN. *In* Forsch.-Ber. über Lebensmittel [etc.], Jahrg. 3, Heft 2, p. 49–54. German abstract *in* Ztschr. Nahr.-Untersuch. [etc.], Jahrg. 10, no. 9, p. 171–173. English abstract *in* Analyst, v. 21, no. 246, p. 233–234. 1896.

[5] HARCOURT, R., and FULMER, H. L. CHEMICAL WORK IN CONNECTION WITH INCUBATION PROBLEMS. *In* Ontario Dept. Agr. Bul. 163, p. 57–66. 1908.

TIME REQUIRED FOR FOOD TO PASS THROUGH THE INTESTINAL TRACT OF FOWLS[1]

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INTRODUCTION

As a preliminary to our study of the digestive coefficients of poultry feeds we wished to run some tests to determine the length of time required for feed to pass the entire length of the digestive tract. The length of the small intestine in a hen of average size is about 61.7 inches, and that of the large intestine 4.61 inches,[2] making a total of 66.3 inches, which, added to the length of the second portion of the esophagus, the proventriculous, and gizzard makes approximately 71.5 inches for the food to pass.

THE PROBLEM

An accurate knowledge of the length of time required for the food to pass the entire digestive tract has a bearing on the time at which it is safe to save excreta from the bird under digestion trial. The problem is to determine just the length of time required for food to pass through the entire digestive tract so that a safe allowance can be made in planning the taking of data of feed given and excreta saved for analyses during the same time.

We also wished to determine whether the rapidity of digestion is the same in growing fowls, broody hens, hens not in laying, and hens in laying.

EXPERIMENTAL METHODS

Fowls of the American breeds were used in these experiments. Only hens were to be tested, and birds 2 to 3 years of age were selected.

The birds were kept in small wire coops 18 inches square with 1-inch chicken netting floor. The coop stood on a tin pan slightly larger than the floor of the coop. This pan was to catch the excreta as it was voided.

Ground feeds, such as wheat middlings and corn meal, were used in which was incorporated a material that would stain the digestive content and indicate just when the food passed out of the body. The indicators were lampblack, methylene blue, and gentian violet.

THE DATA

TEST NO. 1.—White Plymouth Rock hen, leg band No. B36, weight, 5.9 pounds.

8 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
2 p. m. First evacuation; a trace of lampblack appeared.
4 p. m. Still only a trace of lampblack appeared. Fed 20 gm. wheat middlings without lampblack.
6 p. m. Evacuation showing excreta decidedly black.
8 a. m. Second day. 20 gm. middlings and water given.
8 a. m. Third day. After 48 hours excreta showed slight tinge of lampblack on the outer surface but none on the inner particles. 20 gm. middlings mixed with water given.
6 p. m. 20 gm. middlings mixed with water given.
8 a. m. Fourth day. After 72 hours excreta normal; no trace of lampblack. Lampblack appeared in 6 hours and disappeared in 72 hours.

[1] Accepted for publication Jan. 16, 1922.

[2] KAUPP, B. F. THE ANATOMY OF THE DOMESTIC FOWL, p. 148, 150, fig. 31, 36, 37. Philadelphia and London, 1918.

TEST NO. 2.—White Plymouth Rock hen, leg band No. 142, weight 6.8 pounds.

8 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. wheat middlings with water and lampblack.
6 p. m. First excreta passed; lampblack stained excreta.
8 a. m. Second day. 20 gm. middlings given.
6 p. m. Fed 20 gm. middlings. Excreta still black.
8 a. m. Third day. 20 gm. middlings given. Excreta nearly normal; only small amount of lampblack on the outside, inside of mass not containing any.
6 p. m. 20 gm. middlings.
8 a. m. Fourth day. After 72 hours excreta normal.

In this test the first voiding of excreta after the feeding of the lampblack was at 6 p. m., or 10 hours. At this time lampblack in small amounts appeared. All lampblack had disappeared at the end of 72 hours.

TEST NO. 3.—White Plymouth Rock hen, leg band No. B10, weight, 6.14 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
1 p. m. First evacuation; excreta black.
2 p. m. Second evacuation; excreta black.
7 a. m. Fourth day; excreta free from black.

Lampblack appeared in 6 hours and disappeared in 72 hours. This test was run with a broody hen.

TEST NO. 4.—White Plymouth Rock hen, leg band No. 473, weight, 5.14 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. middlings, no lampblack. No excreta were voided on the first day after feeding with lampblack.
7 a. m. Second day. First evacuation; excreta black.
7.30 a. m. Second evacuation; excreta black. This bird acted as though constipated, since no further excreta were voided till morning of the fourth day, or 72 hours. No lampblack was present in these last excreta.

Owing to the fact that no excreta were voided on the day of feeding of the middlings and lampblack, it is impossible to tell how long it required for the lampblack to pass the entire length of the digestive tract. The fact that the excreta were free from lampblack on the fourth day and after the 72 hours showed this test to be in line with those above. This test was run with a broody hen.

TEST NO. 5.—White Plymouth Rock hen, leg band No. B148, weight, 6 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
4 p. m. Fed 20 gm. middlings.
7 p. m. Fed 20 gm. middlings.
9 p. m. First excreta voided; excreta black.
7 a. m. Fourth day. Excreta after 72 hours normal; no lampblack.

This test was run on a broody hen. First excreta voided in 14 hours and showed lampblack. Lampblack traces disappeared after 72 hours.

TEST NO. 6.—Single Comb White Leghorn hen, in laying condition, leg band No. 34, weight, 2.8 pounds.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
10.30 a. m. First excreta voided; excreta black.
11.20 a. m. Excreta voided; same color as at 10.30 a. m.
7 a. m. Fourth day. After 72 hours excreta normal.

Excreta showed in 3.5 hours that food had passed the entire length of the intestinal tract.

TEST NO. 7.—Chick, leg band No. 32, weight, 2 pounds.

7 a. m. Fed 10 gm. wheat middlings mixed with water and lampblack.
10.50 a. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.

The indicator suggests the food passing through this 2-pound broiler chick in 4 hours.

TEST NO. 8.—Chick, leg band No. 36, weight, 1.8 pounds.

7 a. m. 20 gm. wheat middlings given mixed with water and lampblack.
10.55 a. m. First excreta voided; trace of lampblack.
11.40 a. m. Second excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.

In this test the excreta voided indicated that the food had passed through the digestive tract in approximately 4 hours.

TEST NO. 9.—Buff Plymouth Rock hen, in laying condition, leg band No. 26, weight, 5.5 pounds.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
10 a. m. First excreta voided; excreta black.
7 a. m. Fourth day. Excreta normal.

The indicator showed that food had passed the entire digestive tract in 3 hours. An egg was laid the first day of the experiment.

TEST NO. 10.—Buff Plymouth Rock hen, in laying condition, weight, 6 pounds, leg band No. 28.

7 a. m. Fed 20 gm. wheat middlings with water and lampblack.
10.05 a. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.

The indicator showed that food passed through the intestinal tract in approximately 3 hours. This hen laid one egg on the first day of the experiment.

TEST NO. 11.—Buff Plymouth Rock hen, weight, 6 pounds, leg band No. 30, in laying condition.

7 a. m. Fed 20 gm. wheat middlings mixed with water and lampblack.
10.00 a. m. First excreta voided; lampblack present.
3.30 p. m. Third day. All trace of lampblack gone.

The hen laid an egg each of the first two days. The indicator showed that food had passed the entire length of the digestive tract in approximately 3 hours. It was noted in the latter part of this experiment that the outside of the fecal mass was black and the inside not colored. This raised the question as to whether lampblack will adhere to the intestinal mucosa and be gradually eliminated by the feces as it passes along. In the small intestines where the food is mixed by the pouring back and fourth process, it evidently would be mixed with the food. With these ideas in mind, it was decided to try some tests with aniline blue dyes, which would stain the feeds but not be in a form to be carried along mechanically as was the lampblack.

TEST NO. 12.—Columbian Wyandotte hen, leg band No. 26, weight, 5.4 pounds.

7 a. m. Fed 5 gm. whole corn soaked in gentian violet water.
4 p. m. Given 20 gm. soaked in gentian violet water.
7.30 a. m. Second day. First evacuation. Excreta watery in consistency and greenish in color, indicating at first a tendency to constipation and later diarrhea.

This test was considered unsatisfactory.

TEST NO. 13.—Columbian Wyandotte hen, leg band No. 28, weight, 5.6 pounds.

7 a. m. Fed 4 gm. whole corn soaked in gentian violet water. There was no evacuation during the day.
8.45 a. m. Second day. First evacuation watery in consistency and greenish in color.

This test was unsatisfactory for same reason as No. 12.

TEST NO. 14.—Columbian Wyandotte hen, leg band No. 31, weight 6 pounds.

7 a. m. Fed 3 gm. whole corn soaked in gentian violet water.
6 p. m. First evacuation, greenish and watery in consistency. The constipating effect was not so great as in the two previous cases.

This test was considered unfavorable and unsatisfactory.

TEST NO. 15.—Columbian Wyandotte hen, leg band No. 32, weight 5.7 pounds.

7 a. m. Fed 10 gm. whole corn soaked in gentian violet water.
9 a. m. Second day. First evacuation. Feces greenish and watery. The hen appeared weak. Again the gentian violet appeared to cause constipation followed by diarrhea, apparently due to irritation of the mucosa of the bowel.

This test was considered unsatisfactory.

TEST NO. 16.—Buff Plymouth Rock hen, leg band No. 26, weight 5.8 pounds.

8.30 a. m. Fed corn meal mixed with methylene blue water.
11.40 a. m. First excreta voided; excreta blue.
7 a. m. Fourth day. After 72 hours excreta normal.

Food passed through the intestinal tract in 3 hours and 10 minutes. There was some irritation. This bird was in laying condition.

TEST NO. 17.—Buff Plymouth Rock hen, leg band No. 28, weight 6 pounds.

8.30 a. m. Fed 35 gm. corn meal mixed with methylene blue water.
11.00 a. m. First excreta voided. Excreta were blue. There was some irritation at this time, evidenced by slight watery condition of the excreta.
8.30 a. m. Fourth day. After 72 hours all traces of the blue had disappeared.

Food passed through the digestive tract in 2 hours and 30 minutes. This bird was in laying condition. There was again evidence of irritation.

TEST NO. 18.—Buff Plymouth Rock hen, leg band No. 30, weight 5.9 pounds.
8.30 a. m. Fed 8 gm. corn meal mixed with methylene blue water.
9.00 a. m. First excreta were voided; only very slight traces of methylene blue could be detected.
4.30 p. m. Second evacuation; excreta blue.
The first passage was in 30 minutes and indicated that if food is given on any empty crop and mixed with methylene blue slight staining of the food in the cloaca may occur in that space of time. The reaction was constipation followed by looseness of the bowels, as with the gentian trials. At the end of 72 hours no trace of the methylene blue could be detected.

TEST NO. 19.—Buff Plymouth Rock hen, leg band No. 32, weight 5.7 pounds.
8.30 a. m. Fed 30 gm. corn meal mixed with methylene blue water.
11.40 a. m. First excreta were passed; excreta blue
8.30 a. m. Fourth day. After 72 hours excreta normal.
The first indication was at 3 hours and 10 minutes. There was again evidence of irritation. The hen was in laying condition, having laid two eggs during the trial.

TEST NO. 20.—Buff Plymouth Rock hen, leg band No. 34, weight 6.1 pounds.
8.30 a. m. Fed 31 gm. corn meal mixed with methylene blue water.
8.30 a. m. Fourth day. After 72 hours excreta normal.
Indicator showed food passed entire digestive tract in 3 hours and 30 minutes. Hen was in laying condition. Methylene blue caused irritation.

TEST NO. 21.—Buff Plymouth Rock hen, leg band No. 36, weight 6.3 pounds.
7 a. m. Fed 7 cc. methylene blue water mixed with corn meal.
7.30 a. m. Second day. First evacuation; excreta blue.
7.30 a. m. Third day. After 48 hours all blue had disappeared.
This bird was not in laying condition. The first effect was that of constipation, the first evacuation being 23 hours after the test meal was given.

TEST NO. 22.—White Plymouth Rock hen, leg band No. 115, weight 6.5 pounds.
7 a. m. Fed 20 gm. corn meal mixed with lampblack.
10.20 a. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.
This hen was in laying condition, laying an egg on each of the three days of the test. The food, as indicated by the lampblack, passed the entire length of the digestive tract in 3 hours and 20 minutes.

TEST NO. 23.—White Plymouth Rock hen, leg band No. 65, weight 5.8 pounds.
7 a. m. Fed 20 gm. corn meal in which was incorporated lampblack.
12.20 p. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.
The first colored excreta were passed in 5 hours and 20 minutes. The hen was in laying condition, laying each day of the test.

TEST NO. 24.—White Plymouth Rock hen, leg band No. 100, weight 6.3 pounds.
7 a. m. Fed 20 gm. corn meal mixed with lampblack.
2 p. m. First excreta passed; excreta black.
7 a. m. Fourth day. Excreta normal.
The first excreta were voided in 7 hours. This hen was broody. Broody hens apparently do not have as many evacuations as other hens and the quantity evacuated is greater, as indicated by this hen.

TEST NO. 25.—Partridge Plymouth Rock hen, leg band No. 112, weight 5.3 pounds.
7 a. m. Fed 20 gm. corn meal in which was incorporated lampblack.
1.20 p. m. First excreta voided; excreta black.
7 a. m. Fourth day. After 72 hours excreta normal.
The first excreta indicating lampblack was voided in 6 hours and 20 minutes.

DISCUSSION

Of the two White Plymouth Rock hens not in laying condition, one passed wheat middlings in 6 hours and the other in 10 hours, or an average of 8 hours.

In the test with one Single Comb White Leghorn, in laying condition, the wheat middlings passed through the intestinal tract in 3½ hours. Of the three Buff Plymouth Rock hens, in laying condition, two passed wheat middlings in 3 hours and the third in 3 hours and 5 minutes. Of the two White Plymouth Rock hens laying during the tests, corn meal passed through the digestive tract of one in 3 hours and 20 minutes

and of the other in 5 hours and 20 minutes, or an average for these two of 4 hours and 20 minutes. An average of the six tests with laying hens showed that food passed the entire digestive tract in 3 hours and 46 minutes.

Of the three White Plymouth Rock hens that were broody, one passed the wheat middlings through the intestinal tract in 6 hours, the second in 24 hours, and the third in 14 hours, or an average for these three of 14⅔ hours. Of two White Plymouth Rock hens in a broody condition, given corn meal, one passed food through the digestive tract in 7 hours and the other in 6 hours and 20 minutes, or an average for the five broody hens of 11 hours and 45 minutes.

Of the two chicks weighing approximately 2 pounds each, one passed the wheat middlings through the intestinal tract in 3 hours and 50 minutes and the other in 3 hours and 55 minutes, making an average of 3 hours and 52 minutes.

Of the two chicks weighing approximately 2 pounds each, one passed the wheat middlings through the intestinal tract in 3 hours and 50 minutes and the other in 3 hours and 55 minutes, making an average of 3 hours and 52 minutes.

Of four Columbian Wyandotte hens not in laying condition, one consumed 5 gm. whole corn soaked in gentian violet water and voided first excrement in 24 hours; the second consumed 4 gm. and passed the first tinted excreta in 25 hours; the third ate 3 gm. and passed the first tinted excreta in 26 hours. From this it is evident that gentian violet causes constipation followed by a looseness of the bowels indicating irritation to the mucosa of the bowel.

Of six Buff Plymouth Rock hens in laying condition, two consumed 30 gm. whole corn soaked in methylene blue water and voided the first tinted excreta in 3 hours and 10 minutes; the third consumed 35 gm. whole corn and voided tinted excreta in 2 hours and 30 minutes; the fourth consumed 31 gm. corn meal and voided tinted excreta in 3 hours and 30 minutes; the fifth consumed 8 gm. corn meal and voided the first tinted excreta in 1 hour and 30 minutes; the sixth consumed 7 gm. corn meal and voided the first tinted excreta in 23 hours.

In these tests there was a tendency for small amounts of methylene blue to cause constipation and larger doses to cause irritation with specks of blood on the semiliquid evacuations. There was only one exception to this tendency and that was the bird that consumed 8 gm. of corn and evacuated in 1 hour and 30 minutes.

SUMMARY

Digestive processes of the fowl are rapid. The greatest rapidity is shown in the laying and the growing fowl, the passage of food requiring on an average 3 hours and 52 minutes for growing fowls and 3 hours and 46 minutes for laying hens. Next in activity comes the adult hen not in laying condition, requiring 8 hours, and then the broody hen, requiring an average of 11 hours and 44 minutes.

It was noted that broody hens behaved the same in the experimental coops as on the nest; that is, the evacuations were fewer and the quantity evacuated each time increased over that of a normal hen.

We have not taken as accurate the fowls in which gentian violet or methylene blue were given, as it exercised influence on the normal function of the intestinal tract.

In all these tests the hens were placed in the coops the day before the trial so that the crop was empty when the test feed was given.

EFFECTIVENESS OF MULCHES IN PRESERVING SOIL MOISTURE[1]

By F. S. HARRIS,[2] *formerly Director and Agronomist,* and H. H. YAO, *Fellow in Agronomy, Utah Agricultural Experiment Station*

INTRODUCTION

The effective preservation of soil moisture is one of the vital problems which confront the dry farmer and the scientist. Any cultural method that has the power of conserving moisture in the soil is a great asset toward solving the dry-farming problem. But the question of the effectiveness of mulches has been much disputed. While in a majority of cases scientists have reported favorable results with the application of mulches, under different conditions the same mulches have not answered the purpose. Indeed, the literature pertaining to this subject presents such varied opinions that it inaugurates complexity into the significance of mulching. The need of a more thorough study of the problem led to the work reported in this paper. Literature on this subject is much scattered, and no attempt has been taken to include all in this article. Only those statements that are closely related to the subject matter have been briefly summarized. The results of the following experiments are tabulated from averages of thousands of measurements.

HISTORICAL REVIEW

OBJECT OF MULCHING

The object of mulching is to preserve a uniform degree of moisture (*36*)[3] and to hold more water in the soil, the effectiveness of which is brought about by diminishing the direct influence of the agencies of evaporation and by retarding the capillary rise of water to the surface (*22, 48*).

EFFECTIVENESS OF MULCHING

The pioneer on mulch work was Wilhelm (*46, 47*), who observed that soils shaded by growing plants contain the least moisture, others covered with stones and other lifeless objects contain most, while bare soils stand intermediate. This has been confirmed by later workers such as King (*20, p. 105*), who stated that mulched soils contained more moisture to the depth of 3 feet than rolled soils, Wollny (*48, p. 854–859*) and Harris and Turpin (14), who found that the effect of mulches was noticeable several feet below the surface of the ground but that the surface foot showed the greatest benefit. Field investigations by Kedzie (*16*) indicate that to a depth of 16 inches cultivated plots had 3 per cent more moisture than naked fallow.

[1] Accepted for publication Oct. 19, 1921.
[2] Dr. F. S. Harris resigned from the directorship on Sept. 1, 1921.
[3] Reference is made by number (italic) to "Literature cited," p. 740–742.

MULCH MATERIALS

Any lifeless object can be used for mulching (*46; 47; 48, p. 854–859*). Warington (*42*) has said that the evaporation from the soil may be considerably diminished by protective coverings, such as stones, earth mulch, farmyard manure, straw, dead leaves, or coconut fiber. Clover hay (*17*), straw (*8*), hay (*10*), seaweed (*33*), shingle edgings (*13, p. 24–26*) sawdust (*13, p. 24–26*) and grass (*17; 34, p. 91–93; 39; 41*) had been used for experimental purposes with favorable results on the yield of crops. It was reported by Shimm (*39*) that barnyard manure was more effective than straw or fresh manure, in that it held more moisture and secured a more uniform moisture content in the soil to the depth of 4 feet. Halsted (*10*) used fresh hay, salt hay, and excelsior for mulching, but he failed to find any appreciable difference in the yield of peppers, egg plants, cucumbers, tomatoes, beets, or beans. Munson (*32*) concluded that shingle edgings were excellent for mulching either apple trees or strawberries, but that a fine dust cover could not be excelled for gooseberries. Harris and Turpin (*14*) found straw mulch to be better than 2-inch cultivation.

DEPTH OF MULCHING

Laboratory experiments on the evaporation of moisture from the soil under the mulches proved that the effectiveness increases with the depth (*9, 24*). Under the field conditions the same does not follow the general rule. Sanborn (*35*) and Cardon (*6*) recommended shallow cultivation in preference to deep cultivation. King (*21*) found the soil below a 3-inch cultivation to be more moist than that below 1.5-inch cultivation, although the third and fourth feet showed reversed results, which are confirmed by Chilcott and Holm (*7*). Shinn (*39*) reported a 3-inch barnyard manure mulch to be more effective than a 5-inch earth mulch, 6-inch straw mulch, or 3-inch fresh manure mulch. Investigations by King (*24*) showed that shallower depths of stirring are to be preferred to the deeper for long-interval cultivations. That a deep mulch maintained during a season of small rainfalls may waste more water than is saved on account of the fact that small rainfalls are wholly retained in the upper layers of the mulch and lost directly and completely to the air, whereas if the mulch has been thin enough to wet through, capillarity would draw a portion of the water downward into the undistubed soil to render effective service when conserved by subsequent cultivation. The work of Burr (*5, p. 53–84*) showed straw mulches, deep cultivation, and shallow cultivation to rank in effectiveness in the order named.

EVAPORATION OF MOISTURE FROM DIFFERENT SOIL CLASSES

As the soil particles diminish in size there is a large increase in the quantity of moisture brought to the surface (15). This is merely due to the surface distribution of water. Meister (*30*) has pointed out that the amount of water retained by soils depends upon the coarseness or fineness of the particles; the less the internal surface of the mass, the smaller will be the proportion of water retained. When fully saturated with water, sandy soils contain 30.4 per cent of water; clay, 38.5; chalk soil, 39.2; loam, 45.4; and garden earth, 76.8. King (*18, p. 197; 19, p. 152*), Loughridge and Hilgard (*26, p. 80–91*), and Lawes and Gilbert (*25, p. 110*) have obtained somewhat similar results.

MOVEMENT OF SOIL MOISTURE

Soils have a great attractive and adhesive force for water (*2*). The retention of water on the surface of soil particles, despite gravitation, is due to surface tension (*3*). The surface of the film of water encircling a soil particle is in an elastic condition exerting a considerable pressure. If the films of water became thicker and heavier, a part of the water would gradually pass out, and if the films became thinner they would acquire the power of absorbing and retaining fresh supplies of neighboring water. Briggs and Lapham (*4*) have attributed the cause of the movement of soil moisture to viscosity, concentration of solution, surface tension, and moisture film curvature. Lynde and Duprè (*28*) believe that osmosis may cause a considerable movement. King (*23*) and Alway and Clark (*1*) have demonstrated the movement of moisture in the soil. Water always tends to move from the wet to the dry soils (*14*). Briggs (*3*), Briggs and Lapham (*4*), Widtsoe and McLaughlin (*45*), and Loughridge (*27*) have shown that the final distribution leaves the most moisture nearest the source of supply and the least farthest away.

EFFECT OF CULTIVATION ON THE TRANSPIRATION OF PLANTS

Slëskin (*40*) and Schroeder (*37*) have determined the water requirement of plants under cultivation by growing beets in cultivated and cement-covered plots. They found the proportion of beet crop grown under cultivation to that which received the cement-covered treatment to be 26.9 to 16.1. At the end of the experiment, the soil under the cement-covered plot contained more moisture than that under the cultivated plot. Experiments by Widtsoe (*43 p. 14–23*) led to the opposite conclusion. The transpiration ratio was invariably smaller on the cultivated than on the uncultivated soils. On the college loam, the ratios on the cultivated and uncultivated plots were 252 and 603; on the sandy clay, 428 and 535; and on the infertile clay, 582 and 750. The favorable effect of cultivation was shown in the great reduction in the water cost of dry matter resulting from simple tillage.

As a general rule, the more water offered the plant the larger the total yield of dry matter (*38*, *44*). Mayer (*29*) found that the yield increases with the increase in soil saturation up to a certain point, after which there is a strong diminution in the yield of dry matter. Harris (*11*) and Morgan (*31*) have proved that the transpiration ratio increases as quantity of water added to the soil increases—that is, the water cost of crops becomes larger as more water is used.

TIME OF TILLAGE

Harris and Jones (*13*, *p. 24–26*) collected moisture samples from the Nephi substation showing that the moisture content of the fall-plowed and spring-plowed plots receiving like treatments was practically same in all cases. The percentage moisture of the spring-plowed plots increased slightly with depth of cultivation, while the fall-plowed plots slightly decreased inversely with depth of cultivation. Straw mulch on the fall-plowed land is the most effective. In comparing fall plowing with spring plowing, Harris, Bracken, and Jensen (*12*, *p. 30–37*) did not find any material difference in the yield of Turkey wheat. Cardon (*6*) has shown

that the 4-year average moisture content was higher in the spring-plowed plots; but the first 2 feet of the fall-plowed plots contained a higher percentage of moisture.

MULCH VERSUS NO MULCH

In order to demonstrate the effectiveness of mulching in preserving moisture in the soil, an experiment was carried out by using sand immersed in water. The sand was of uniform physical structure, passed through a 100-mesh sieve. It was placed to the depth of 3 inches in a round can, 6 inches in diameter. The bottom was perforated, so that when the can was immersed in water, the soil could be completely saturated through its capillary system. The experiment consisted of free water surface, bare sand, and a 1-inch straw mulch. Each set was performed in triplicate to eliminate experimental error. The experiment extended from April 5 to May 7, and during these 33 days the loss of moisture is recorded in Table I.

Taking the moisture lost from the bare sand as 1,000 per cent, then that which is lost from the soil under the 1-inch straw mulch amounts to 40 per cent. In fact, between the free water surface and bare sand there is only a difference of 8 per cent in favor of the latter. Thus it is to be concluded from this experiment that a 1-inch straw mulch, otherwise under identical conditions, is capable of preserving 60 per cent more moisture in the soil than without mulching.

TABLE I.—*Loss of moisture during the 33-day test*

Can No.	Free water surface.	Bare sand.	Straw mulch.
	Cc.	*Cc.*	*Cc.*
1	1,425.3	1,160.5	480.4
2	1,323.7	1,144.3	439.7
3	1,404.2	1,102	437.9
Average loss	1,384.4	1,135.6	452.7

EFFECTIVENESS OF DIFFERENT MULCH MATERIALS

With the effectiveness of mulching ascertained, the question of the relative effectiveness of mulch materials naturally arises. Almost any lifeless material can be used for mulching, but the relative effectiveness of the different mulch materials varies according to their physical and chemical properties. For the purpose of comparison, five kinds of mulch materials were used—fresh manure, wood shavings, grass, hay, and straw—all maintained uniformly at the depth of 1 inch.

It is seen from Table II that so far as the conservation of moisture is concerned, straw is by far the most effective, and then come hay, grass, wood shavings, and manure, in the order of their effectiveness. The difference between the first and the last is 54.1 per cent; hence the importance in choosing the proper type of mulch material. Such a profound difference demands an elucidation. It was observed that the straw was the most loose and open, whereas the manure mulch formed a uniform and compact covering. When the experiment was over, the mulches were removed and their moisture content determined (Table III).

TABLE II.—*Loss of moisture during 33-day test*

Can No.	Manure.	Wood shavings.	Grass.	Hay.	Straw.
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
1	1,004.4	825.0	679.5	674.1	480.4
2	980.7	782.6	665.2	661.1	439.7
3	980.5	772.8	668.6	656.3	437.9
Average loss with manure as 100 per cent	987.5	793.5	670.4	663.8	452.7

TABLE III.—*Percentage of moisture content of mulches*

Manure.	Shavings.	Grass.	Hay.	Straw.
144.7	23.2	22.7	20	11.8

A comparison of Table III with the foregoing will show that there is a correlation between the moisture lost from the soil and the moisture contained in the mulches. The moisture content of the mulches varies from 144.7 per cent for the manure to 11.8 per cent for the straw, and the total moisture lost from the soil varies accordingly. That is to say, the more absorptive the mulch the more moisture is lost from the soil. Since the purpose of a mulch is to check the soil surface from exposure to the physical agencies of evaporation, the less absorptive the mulch the less water is brought up to the surface to be exposed and therefore the more moisture is held in the soil. Due to the large amount of colloid matter present in the manure, it is capable of absorbing water one and one-half times its own weight, while the dry straw absorbs only one-tenth of its weight. With different quantities of water distributed in approximately equal volumes of manure and straw, it stands to reason that there should be more exposed by the former than by the latter. The same principle can be applied to the intermediate mulches.

However, the preceding tables do not show a reasonably close correlation between the moisture lost from the soil and the moisture content of the mulches, which tends to show that there must be some other factor or factors which exercise a certain degree of influence upon the effectiveness of the different mulch materials. That the effectiveness of mulch materials is materially influenced by their capillary system is proved conclusively by a similar set of mulches suspended in wire gauze. The mulches, otherwise the same, were suspended about ¼ inch above the soil surface, so that the direct capillary system between the soil and the mulches was severed. The results are shown in Table IV.

TABLE IV.—*Loss of moisture through mulches in contact and mulches in suspension, illustrating the action of capillarity*

	Shavings.	Hay.	Grass.	Manure.	Straw.
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
Mulches in contact	793.5	663.8	670.4	987.5	452.7
Mulches in suspension	431.5	392.2	471.2	785.2	387.8
Difference	362.0	271.6	199.2	202.3	64.9
Percentage of loss due to absorption	53.6	59.1	70.3	79.5	83.7
Percentage of loss due to capillarity	46.4	40.9	29.7	20.5	16.3

The word "absorption" as used in Table IV is construed to mean more than the retentive power of the mulches, but includes any water that has escaped through the mulches other than by the capillary action. The difference is brought about by the severence of the capillary system between the soil surface and the mulches, or, in other words, it is the total moisture lost minus that which is evaporated through the capillary action of the mulches. It may be noted that in every case there is more

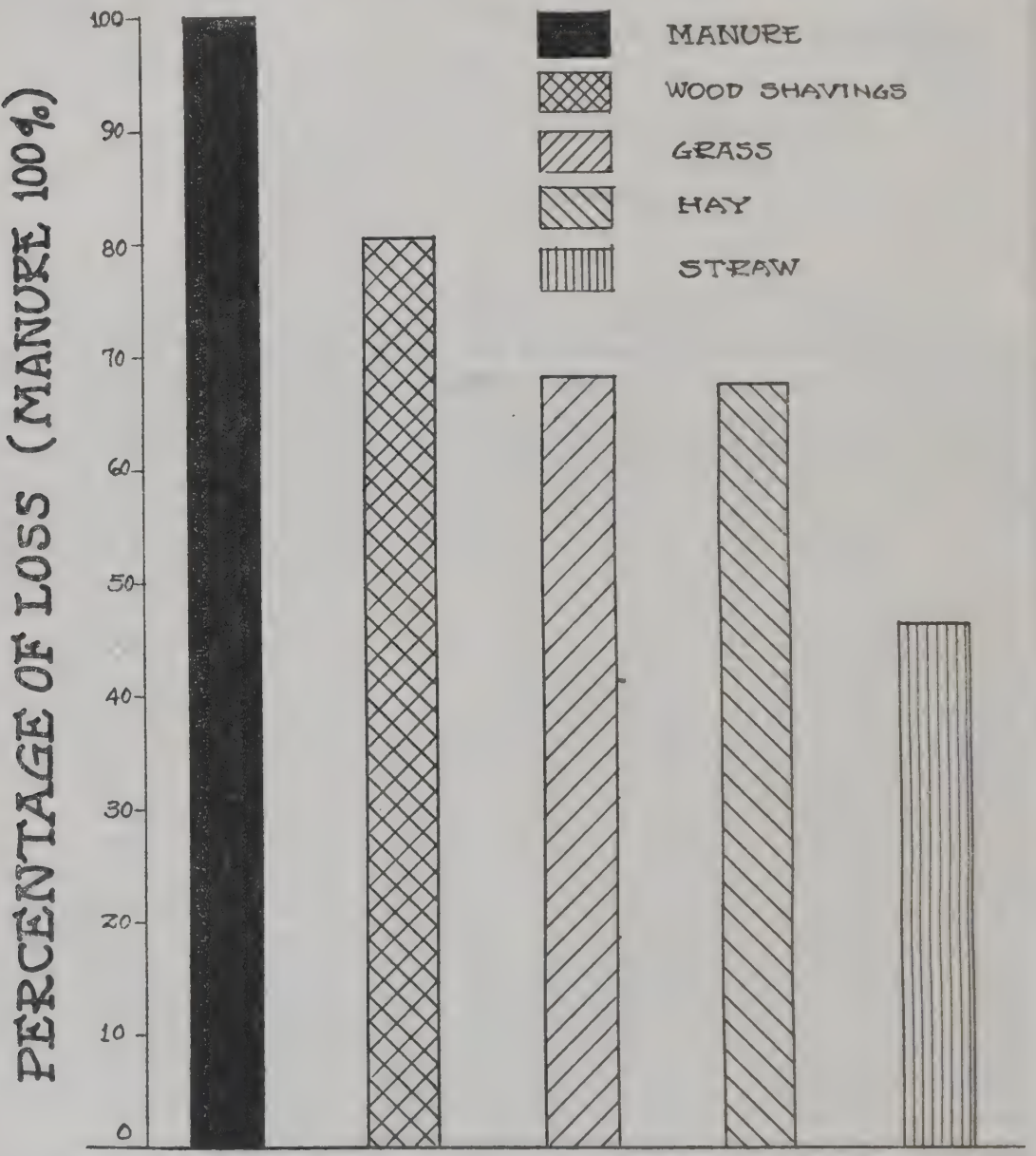

FIG. 1.—Diagram showing the relative losses of moisture from sand with the application of different kinds of mulch materials.

loss due to absorption than to capillarity, although with the wood shavings the difference is trifling. From what has been said, the general rule may be laid down safely that an effective mulch must be constituted of that kind of a material which does not absorb or retain moisture readily and which forms practically no capillary system in itself. A mulch material like manure, which is very porous and therefore holds a good deal of moisture, should not be allowed to stand on the soil except in wet seasons, but must be turned under and thoroughly mixed with the soil before the dry season starts in, otherwise excessive evaporation is likely to take place and the fertility of the manure will deteriorate.

DEPTH OF MULCHING

The depth of mulching has just as much bearing upon the soil moisture as the mulch material. Experiments were carried out with straw and sawdust in galvanized tubes, 36 inches tall, all having the same diameter of 3.5 inches. The bottom of the tubes was perforated to allow free passage of water with the aid of the capillary action of sand. The depths tested were 1 inch, 2, 3, and 4 inches. Water measurements were taken on May 31 and were followed up at various intervals until June 28.

TABLE V.—*Loss of moisture during 60 days with different depths of mulches*

Depth.	Sawdust.	Straw.
Inches.	*Cc.*	*Cc.*
1	309	250
2	173	162
3	110	112
4	104	109

During the same period the unmulched or bare sand lost 531 cc. of water. This will serve to illustrate the point that the efficiency of a mulch increases with its depth. In both cases more water is saved when the depth is increased. Between sawdust and straw, when the depth is beyond 2 inches, there does not seem to be any material difference in the amount of moisture lost, but with the 2-inch and especially the 1-inch mulches, straw is more efficient.

MULCH ACTIVITY UPON DIFFERENT SOIL CLASSES

As the soil particles diminish in size, and therefore increase in surface, more water is brought to the surface. Soils that contain any appreciable amount of porous bodies, hydrates, or colloid matter have a larger water-holding capacity. Since the rate of evaporation is proportional to the moisture content and the extent of exposure of the soil particles, different classes of soil naturally will evaporate variable quantities of moisture.

This subject in question is studied in connection with mulches. Duplicate samples of sand, loam, and clay were mulched with 1 inch of hay.

TABLE VI.—*Loss of moisture during 55 days from different classes of soils under 1-inch straw mulch*

Sand.	Clay.	Loam.
Cc.	*Cc.*	*Cc.*
736	759	846

The same principle seems to govern the evaporation ratio of these three classes of soils. Sand, being much coarser than either clay or loam, lost the least quantity of water. Loam lost the most moisture because its physical structure is much finer and yet passage of water is not

interfered with. Clay has the finest particles, and accordingly it might have been expected to lose the most moisture. But the clay used in this experiment had been ground into powder in the laboratory, and the particles were so fine that the freedom of the movement of water was retarded and therefore evaporation was checked.

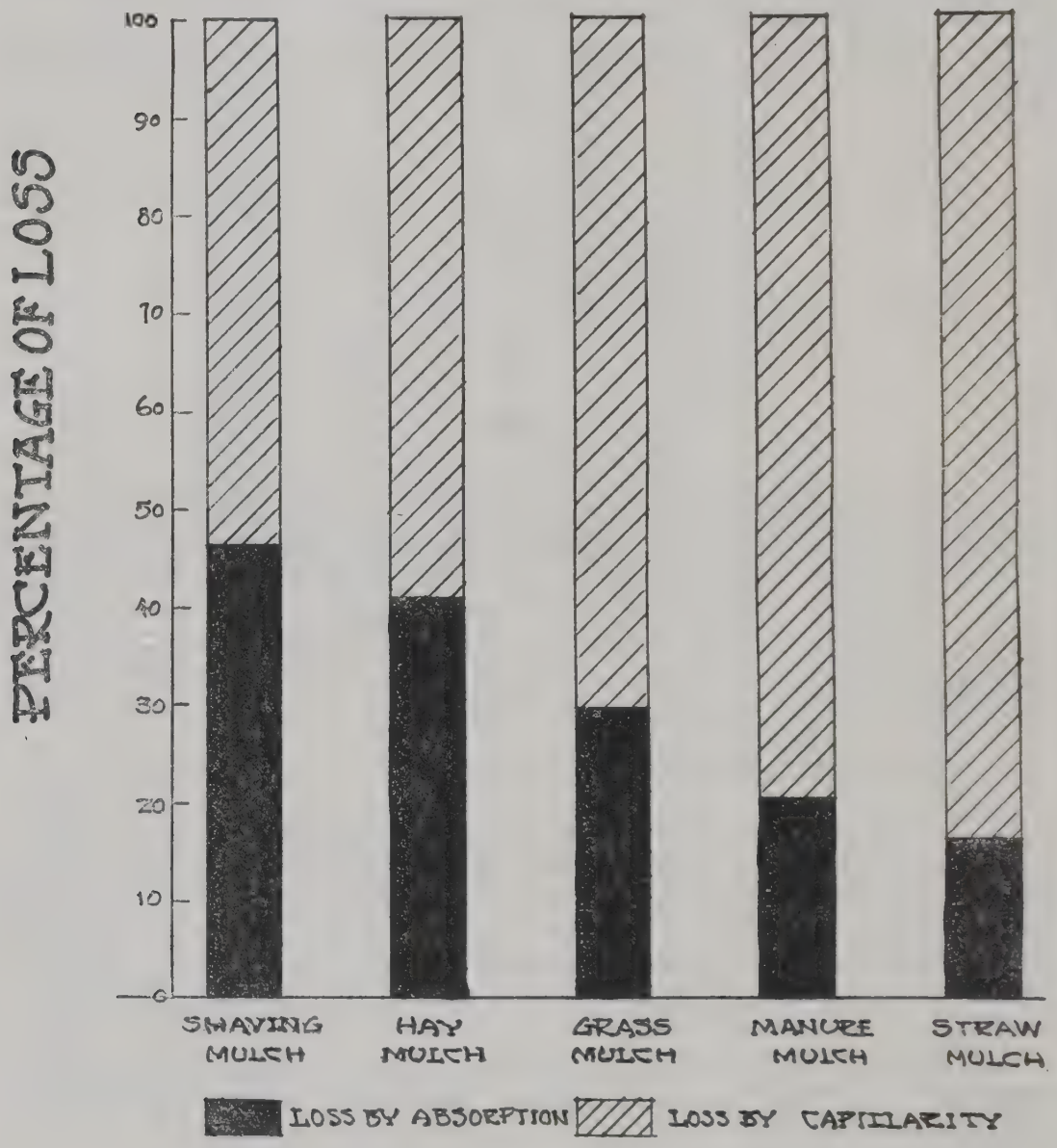

FIG. 2.—Diagram showing the loss of moisture due to absorption and capillarity, taking the total loss as 100 per cent.

EVAPO-TRANSPIRATION RATIO AS AFFECTED BY MULCHING AND CULTIVATION

In so far as mulching or cultivation has the power of preserving moisture in the soil, what effect do they have upon the transpiration of plants? Do the plants make use of the water economically with mulching or cultivation?

The apparatus designed for this experiment consisted of a soil can with a water tank connected at the bottom. Water from the tank was allowed to run into the soil can, and it in turn was brought up to the surface by the capillary action of the soil. Measurements of the water surface were made twice a week by means of an evaporation gage

micrometer. The soil surface had an area of 7.07 square inches through which moisture could be lost.

Six seeds of Long White Icicle radishes were planted in each set of apparatus, of which four germinated in the can marked "no mulch" and "cultivation twice a week," and five in the can marked "cultivation once per week" and "1-inch straw mulch." Seeds were sown on June 23, and the experiment was terminated on July 23. Cultivation was performed by scarifying the surface inch of soil.

The saving of water is a little over 2 inches greater with cultivation than without, and the effectiveness of cultivation in preserving soil moisture increases with the extent of the cultivation. The 1-inch straw mulch saves even more moisture than does cultivation. The effect of cultivation and mulching upon the growth of the radishes needs con-

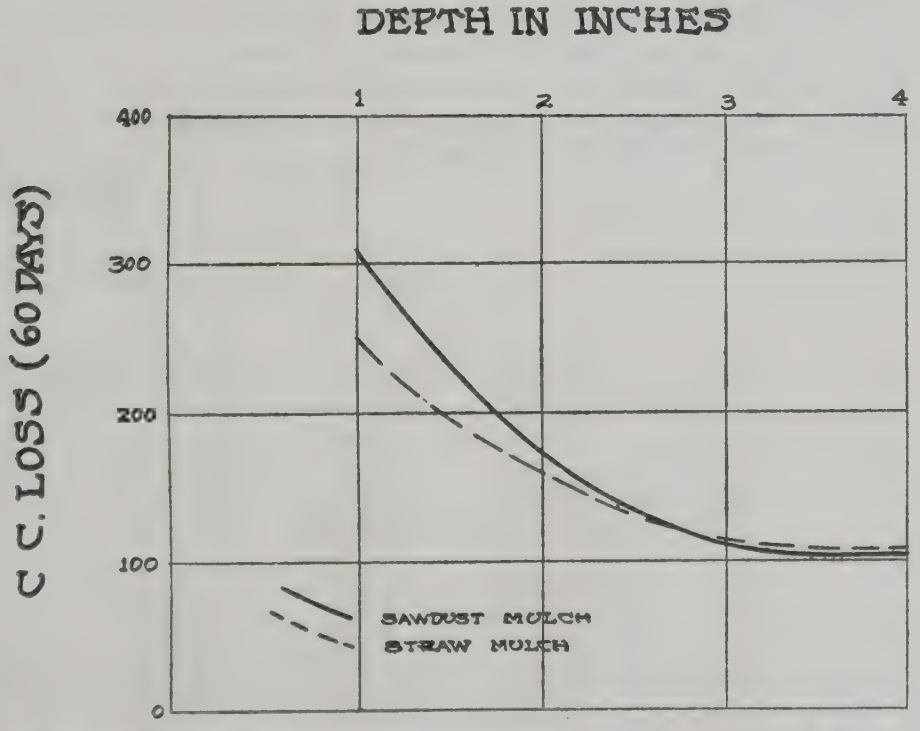

FIG. 3.—Diagram showing the efficiency of mulches with the increase in their depths.

sideration. Without mulching or cultivation radishes are vigorous and the roots uniform, while with cultivation once or twice a week the plants are not so thrifty and the growth of roots is inclined to be stunted. With the 1-inch straw mulch the growth is medium, but the roots are slighlty shorter and smaller than those grown without mulching. The water cost of one part of radishes on the dry-weight basis is, without cultivation or mulching, 1,009; with 1-inch straw mulch, 1,045; with cultivation once a week, 1,422; and with cultivation twice a week, 2,262. In other words, cultivation and mulching save more moisture, but without cultivation the plants make the most thrifty growth.

So far as the conservation of moisture is concerned there is no question that cultivation and mulching are beneficial. The fact that with cultivation and mulching the plants do not grow vigorously is due to three unfavorable conditions.

First, there is too much water within the root zone, the water table being only 15 inches below the surface. The water has been accumulated to such an extent that it is far beyond the optimum moisture content of

the soil. This, indubitably, has a tendency to shut off the air from the roots, which is essential to their proper growth. As it is, they can not penetrate deeply, which seems to be the main causes for their growth's being stunted.

Secondly, cultivation cuts off the root hairs; thus the normal function of the tap root is much handicapped. Frequent cultivation, once or twice a week, does not give the tap root enough time to build up its new root hairs.

Thirdly, frequent cultivation renders the soil too loose, thereby breaking up the contact of the roots with the soil. Since the difference between the soil surface and water is only 15 inches, and the soil, being partially saturated, is already loose enough to induce a favorable root expansion, constant stirring of the soil simply destroys the necessary degree of compactness around the root.

Under field conditions in the dry-farming regions where the precipitation is not over 20 inches annually, there is no chance of ever saturating the soil for any length of time. Cultivation or mulching can be recommended only where water becomes the limiting factor to crop growth. If there is enough precipitation to warrant a good and vigorous growth, or if the water table is near the surface, cultivation or mulching may be entirely out of place.

TABLE VII.—*Evapo-transpiration ratio of radishes as affected by mulching and cultivation*

Treatment.	Evapo-transpiration.					Weight of radishes.			Evapo-transpiration ratio (parts of water to 1 part of radishes by weight).
	Germination stage.	Seedling stage.	Root stage.	Total.		Tops.	Roots.	Total.	
				Inches.	Kgm. equivalent.				
	Inches.	*Inches.*	*Inches.*			*Kgm.*	*Kgm.*	*Kgm.*	
No mulch	3.629	4.212	5.292	13.133	99.349	0.0251	0.0733	0.0984	1,009
Cultivation once a week	3.526	3.654	3.895	11.075	83.777	.0205	.0384	.0589	1,422
Cultivation twice a week	2.771	3.623	3.415	9.809	74.207	.0160	.0168	.0328	2,262
1-inch straw mulch	2.538	2.988	3.793	9.319	70.526	.0187	.0488	.0675	1,045

SOIL MOISTURE AS AFFECTED BY TIME OF CULTIVATION

The question of spring or fall plowing has been one of the dry-farming problems. Opinions seem to differ. An experiment was carried out on the Nephi substation on $\frac{1}{10}$-acre plots to determine the moisture content of the soil by spring and fall plowing and by the different depths of cultivation.

TABLE VIII.—*Average soil moisture percentages to the depth of 6 feet with weeds pulled and the different depths of cultivation under spring and fall plowing*

	Spring plowing.	Fall plowing.
Weeds pulled	18.7	20.2
2-inch cultivation	19.4	20.5
4-inch cultivation	20.4	21.4
6-inch cultivation	19.4	20.0

Moisture samples taken from the Nephi substation to the depth of 6 feet prove in every case that fall plowing preserves more moisture than spring plowing. The 4-inch cultivation is more efficient than the 2-inch, but the 6-inch cultivation drops off a little. The table also shows that cultivation is better than clean culture.

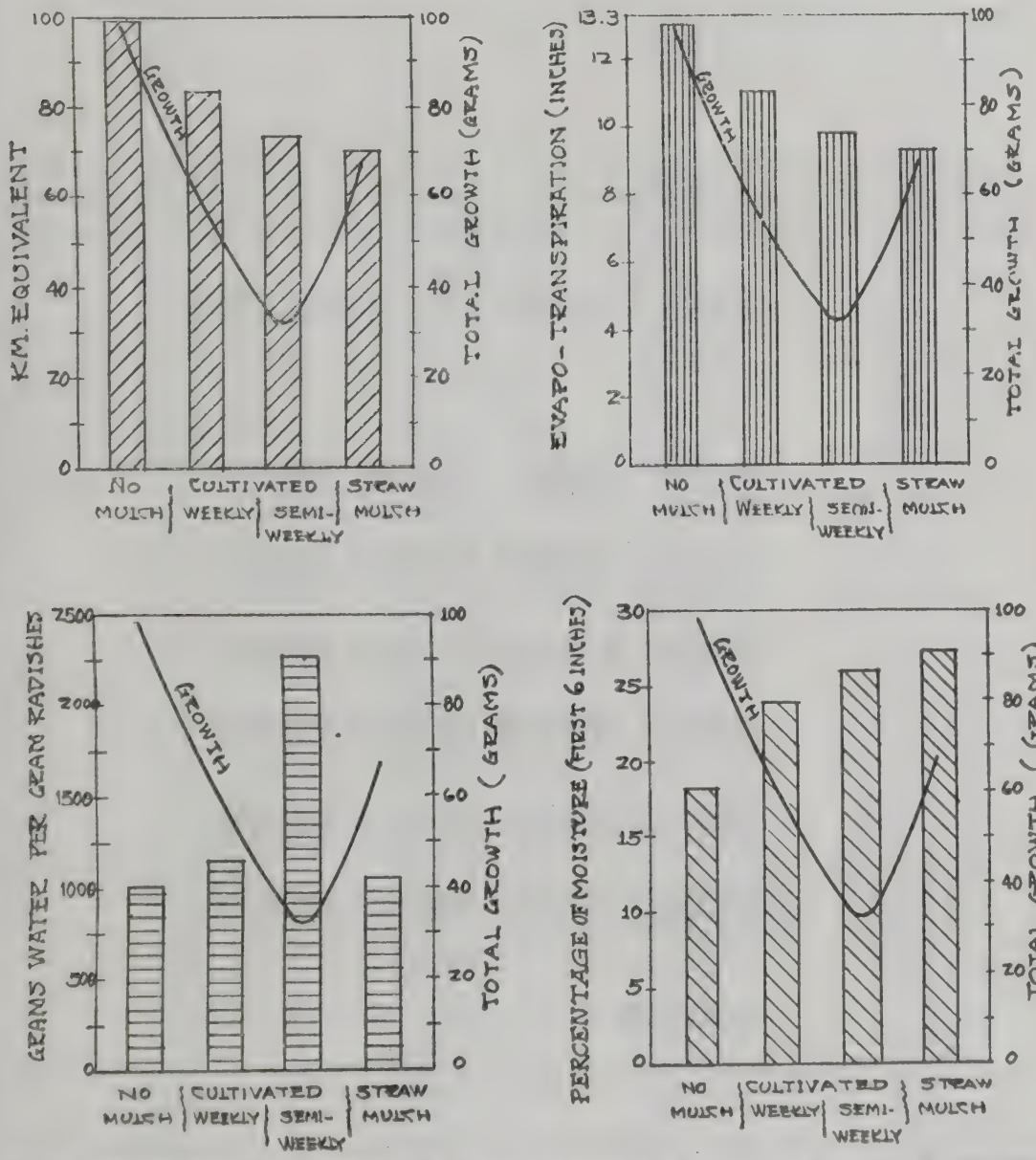

FIG. 4.—Diagram illustrating the growth of radishes as affected by cultivation, frequency of cultivation, straw mulch maintained at the depth of 1 inch, or no treatment, and also the percentage of water in the surface 6 inches of the soil.

MOVEMENT OF SOIL MOISTURE UNDER MULCHES

When soils of different moisture contents come into contact there is a movement of moisture from the thick films of water around the soil particles to the thin films, until equilibrium is reached. That is, water tends to move from the wet to the dry soils, and the influence of cultivation can be proved indirectly by tracing the direction in which the soil moisture moves. At the Nephi substation, $\frac{1}{10}$-acre plots were set aside for mulch experiments. The adjoining strip of land was neither mulched nor cultivated. Four borings to the depth of 6 feet were taken 23 feet apart, from the middle of the plots to the adjoining unmulched land.

TABLE IX.—*Average moisture percentages to the depth of 6 feet, showing the movement of water under mulches*

	Middle of plot.	23 feet from middle.	46 feet from middle.	Unmulched land.
Fall-plowed, straw mulch 4 inches.....	24.7	23.0	21.7	21.0
Fall-plowed, earth mulch 4 inches.....	21.4	21.4	21.2	21.0
Spring-plowed, earth mulch 4 inches.....	21.4	21.4	19.4	19.4

The highest moisture percentages are found in the middle of the plots. Moisture percentages of the soil decrease as they approach the unmulched land, and the lowest are found in the unmulched land. If there is no move-

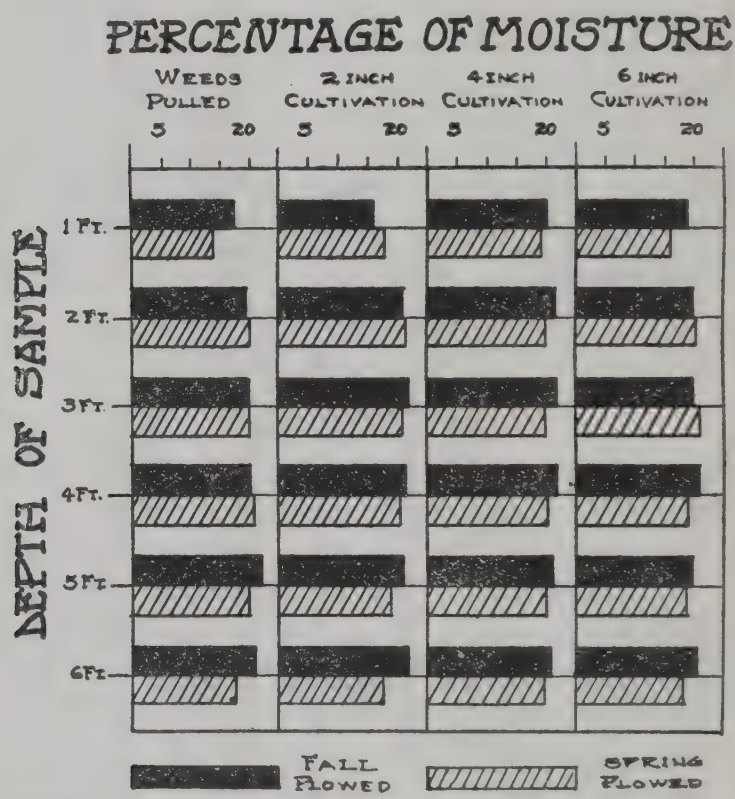

FIG. 5.—Diagram showing the retention of soil moisture as influenced by clean culture and the different depths of cultivation to the depth of 6 feet in the fall-plowed and spring-plowed plots.

ment of water, then there should be the same amount of water anywhere under a uniform mulch. The fact that there is more moisture in the middle of the plots and less moisture as it approaches the adjoining unmulched land shows plainly that water moves out from the mulched plots to the unmulched land. Under laboratory conditions, where side leaching and deep percolation of water can be controlled, the effectiveness of mulching can be determined easily. But under the field conditions, the same factors can not be controlled. Whatever water may be saved by mulching may be lost through either of the foregoing processes. Thus it is not uncommon to find that a mulched plot may not have any more moisture than the unmulched, or that a thicker mulch may not be so effective as a thinner one. As shown in Table VIII, the 6-inch cultivation, either spring-plowed or fall-plowed, is not so efficient as the 4-inch. It is very probable that when the mulch is kept too thick, light rainfalls

can not wet it thoroughly, and since its capillary system with the subsoil is not connected up, no water can be drawn downward. The water is being held in the upper layers of the mulch and then lost to the air

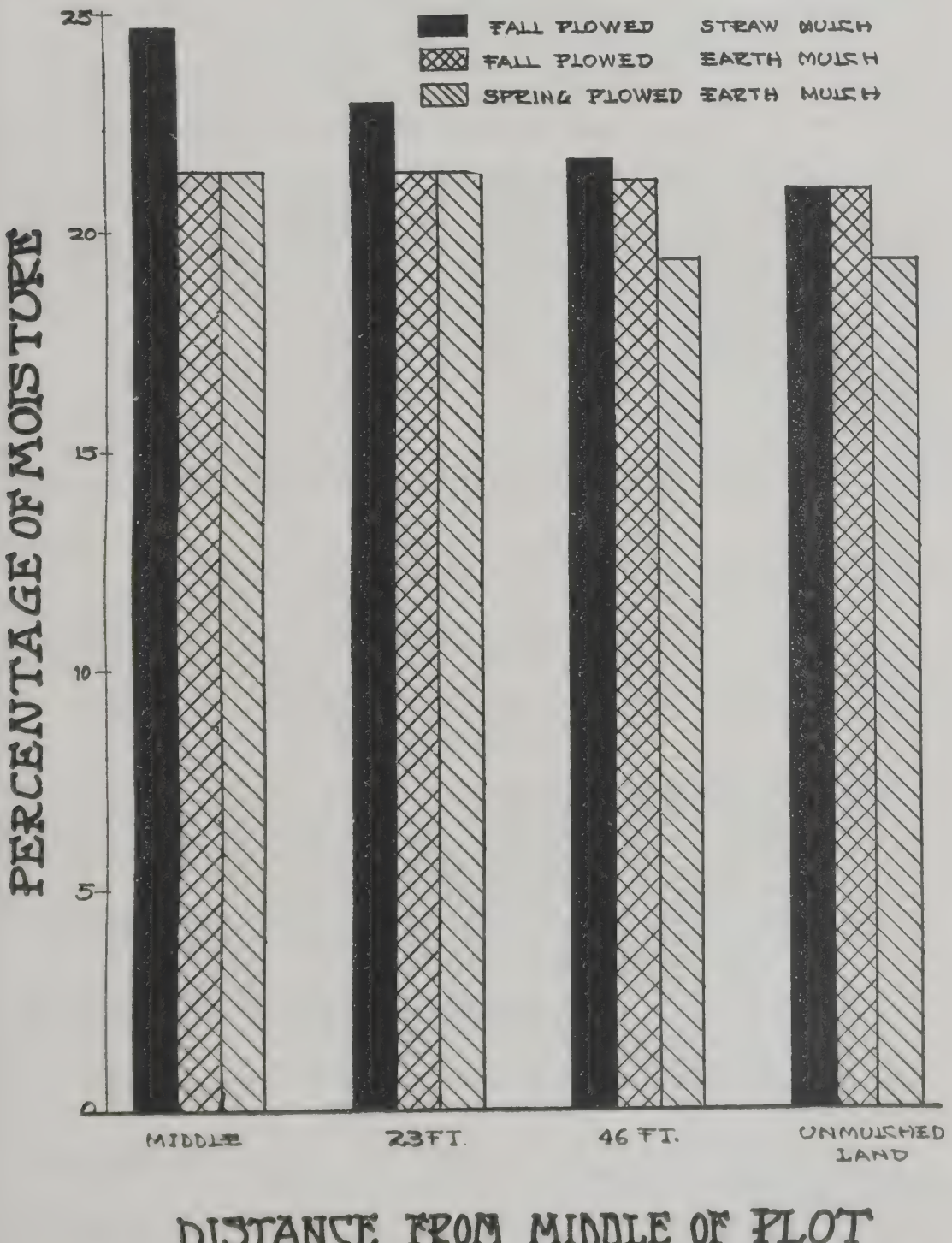

FIG. 6.—Diagram showing the gradual decreases of moisture in the soil to the depth of 6 feet from the mulched plots to the adjoining unmulched land to illustrate side leaching.

directly and completely. At Nephi, or elsewhere in the dry-farming regions, where the annual precipitation is low and only a small part of it comes during the summer months, shallower cultivation is to be preferred to the deeper.

SUMMARY

(1) The question of the effectiveness of mulching has been much disputed. Further study is desirable. The results of these experiments are reported in terms of averages from thousands of measurements.

(2) An effective mulch of 1 inch of straw is capable of preserving 60 per cent more moisture in the soil than is retained without mulching.

(3) Straw is the most efficient mulch material used in the experiments. Then come hay, grass, wood shavings, and manure, in the order mentioned.

(4) The loss of moisture from the soil is correlated with the percentage of moisture retained by the mulch. An efficient mulch must be constituted of material which does not absorb or retain moisture readily and which forms practically no capillary system in itself. An absorptive mulch can be used to an advantage provided it is turned under before the dry period begins.

(5) The effectiveness of mulching and cultivation increases with their depths.

(6) The rate of evaporation of soils under mulch varies according to their moisture contents. Finer soils lose more water. Clay that has been ground into a fine powder in the laboratory loses less water than loam.

(7) Cultivation and mulching save more moisture, but the evapotranspiration ratio is the least with no mulch or cultivation.

(8) Fall plowing preserves more moisture than spring plowing.

(9) Under field conditions, soil moisture is more variable. The 6-inch cultivation is not so efficient as the 4-inch. In the dry-farm regions, shallower cultivation is to be preferred to the deeper.

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ORIGIN OF THE CENTRAL AND OSTIOLAR CAVITIES IN PYCNIDIA OF CERTAIN FUNGOUS PARASITES OF FRUITS[1]

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SCOPE AND PURPOSE OF THE INVESTIGATION

An investigation of fungi associated with rotting strawberries and dewberries during shipment and marketing has afforded an opportunity for study of the development of the pycnidial form of several species of fungi, from which it appears that in these forms there are three principal stages or periods of growth. The first is one of rapid cell multiplication whereby a certain amount of fundamental or undifferentiated tissue is formed with which to begin the construction of the fruit body. The second or intermediate stage includes those activities leading to the formation of the central cavity, the organization of the wall, the delimitation of sporogenous tissue, and, in many forms, the construction of the ostiole. Previous study has largely been confined to the third stage, which includes spore production and other developments which characterize the mature pycnidium. The methods by which the plectenchymatous primordium is formed are also fairly well understood, but just how the characteristic pycnidium is evolved out of the mass of undifferentiated tissue has not been clearly explained. The intermediate stages in the growth of phycnidia of several species have been followed by the writer, and the results of the studies on three of these are reported in this paper.

Bauke's contribution (*2*)[2] is noteworthy because of the clearness with which he describes the two different methods by which pycnidia originate; and on this point, which was his main contribution, his work has been repeatedly confirmed. From the very nature of the subject of his investigation, the method which he employed was not adequate to give the best results. He found it difficult to learn what takes place in the pycnidial "knot" when the cavity is being formed and the sporiferous layer organized, as he was compelled to study the fungus as it grew on slides. In the pycnidium of *Cucurbitaria elongata* a cell at the center becomes divided into a few radially arranged pyramidal cells with their apices together at the center. These are the sporophores or their mother cells. They divide again in various directions or at once cut off spores. By the gelatinization of their walls, the sporophores are separated from each other to form the cavity, which is further increased by the swelling of the mucilage derived from the walls of the spores. In the pycnidium of a Diplodia on Cornus there is at the end of the first period of growth a

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[2] Reference is made by number (italic) to "Literature cited," p. 758–759.

pseudoparenchymatous structure of thin-walled cells bordered by a cortical region. Now at various points in the central portion there appear elliptical areas marked by inwardly growing hyphæ. There are a number of these regions of growth corresponding to the number of locules which will appear in the young pycnidium; some of them may become united as the phycnidium ages. According to Bauke it is the head-on thrust of these young sporophores against each other and the pressure due to the gelatinization of their walls that accounts for cavity formation by forcing the sporogenous layer farther and farther out, crushing the tissue between it and the cortex so that the protoplasm of this crushed tissue can be used in nourishing the spores.

De Bary (*3*), without referring to Bauke's account, describes the development of the pycnidium of a species of Pleospora and says that the central cavity is due to the failure of the cells at the center of the pycnidial primordium to keep pace in their growth with those in the periphery so that the cells at the center are torn asunder. Such a cavity would be schizogenetic.

Baccarini (*5, p. 69*) describes the formation of a cavity in *Sphaeropsis malorum* and adds a further note on the development of pycnidia in general (*6, p. 150–151*). Certain cells at the center of the pseudoparenchyma can be distinguished from the rest by their abundance of oil and glycogen. Their walls become transformed into mucilage which, absorbing water, swells to form a central, oval, or spherical cavity lined with sporophore fundaments. This sporiferous layer plays an important part in the further development of the pycnidium. Baccarini does not say that the entire contents of the cells at the center disappear as the cavity is formed. His account is very similar to that given by Bauke, adding nothing except to state (*6, p. 150*) that lysigenetic and schizogenetic factors enter into the processes, depending upon the quantity of tissue present at the center of the primordium and on the rapidity with which it is reabsorbed.

Reddick (*8*) studied the development of the pycnidium of blackrot of the grape. He states that the activity at the center of the "gnarl" is evidenced by the more deeply staining contents of the cells. He finds that a number of hyphae grow inwardly and assumes that these are the young sporophores. He realizes that the cavity later increases in size, and he believes that it does so in some way at the expense of the pseudoparenchyma. In his description of the origin and the development of the perithecia the nature of the "sclerotium" is discussed. He believes that such a body develops into a perithecium and gives it the name pycnosclerotium.

Hesler (*10*) describes briefly the development of the pycnidium of *Sphaeropsis malorum*. His account is similar to that given by Baccarini, to which he refers. Hesler, however, states without further comment or figures that the cavity is formed by the breaking down of cells and that the ostiole is formed in the same way. He is of the opinion that the dome-shaped tissue (often shown in figures of this pycnidium) from which the sporophores arise is due to irregularities in the cavity which is tending to become multilocular.

In order to best illustrate the relative importance of the two factors, disorganization and growth inequalities, in cavity formation, the intermediate stages in three pycnidia will be described. The formation of the ostiole will be considered in connection with one of the forms.

BLACKROT FUNGUS OF DEWBERRY

There is a blackrot fungus of dewberries the pycnidia of which are very similar to those of *Phyllosticta paviae*, *P. labruscae*, and *P. solitaria*. Pycnidia frequently develop on berries from North Carolina found in the New York and Washington markets. It requires but a short time for the black rot to make its appearance if berries are placed in damp chambers. On account of the slowness with which the disease develops in transit and market it will probably not prove of much economic importance.

The fungus has not been connected with its perfect stage, but it may very well belong to *Physalospora carpogena* Atkinson, authentic specimens of which apparently are not in existence. The pycnidial stage has therefore been called by Shear *Phyllostictina carpogena*, and a formal description is to be published.

The fungus has been isolated, and pycnidia have been grown to maturity on agar media. Dewberries artificially infected with spores from these cultures have developed the typical blackrot. An abundance of material from natural sources and from these cultures has been available for study.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The "gnarl of hyphae" develops into a plectenchymatous body which has been called a "pycnosclerotium" (Pl. 1, A), such as has been noted and described many times by those who have studied the blackrot of the grape. The large outer cells appear dark in color with thick walls, but toward the center the cell walls are very thin. The central portion possesses sufficient food for future structural growth processes. While in this fungus, as well as in that of grape blackrot, it is undoubtedly true that similar bodies are immature spermogonia, or young perithecia, there can be no question that every pycnidium of both species passes through some such form in its development. If cavity formation for some very definite reason begins early, while the primordium is still plastic, then the resemblance to a "sclerotium" is not so marked. These bodies are frequently as large as mature pycnidia. If in such case there is little further enlargement, how may we account for the central cavity of the pycnidium? In the sections shown in Plate 2, A, B, and Plate 1, B, there is evidence of the beginning of disorganization of cells at the center. As soon as one or two cells disintegrate sufficiently, the surrounding ones push into the mucilaginous matter and in their turn begin to disorganize. It may be that degeneration is at first due to the head-on thrust of cells pushing inwardly; but, if one may judge by what follows, it is more likely that the breaking down process begins first.

The cells of the next succeeding layer to push into the small open space present the appearance of rather thick sporophores, which is misleading (Pl. 2, C, D; 1, C), since ordinarily these also become disorganized by degrees, and the cells below them push out in their turn. The wall layers are not as yet well organized. The conditions in this stage are shown in Figure 1, *a*. The cells lining the cavity clearly result from the budding or division of the cells of the original pseudoparenchyma. They do not show in this section very decided effects of pressure from within, such as Bauke (*2*) says results in crushing and flattening the inner-wall cells. Later, as the cells elongate or even divide tangentially, this

appearance is more exaggerated. From each of these cells two or three hyphae now branch out, each composed of one to several cells, the outermost being in various stages of disorganization. The nuclei are quite distinct; frequently there are two lying close together in each cell. So similarly do the cavities in pycnidia and spermogonia originate that one can not be certain into which such a structure will eventually develop. These protosporophores occasionally produce spores, since a few pycnospores sometimes are found in sections of such young pycnidia, just as spores are rarely budded off directly from the thin-walled cells at the

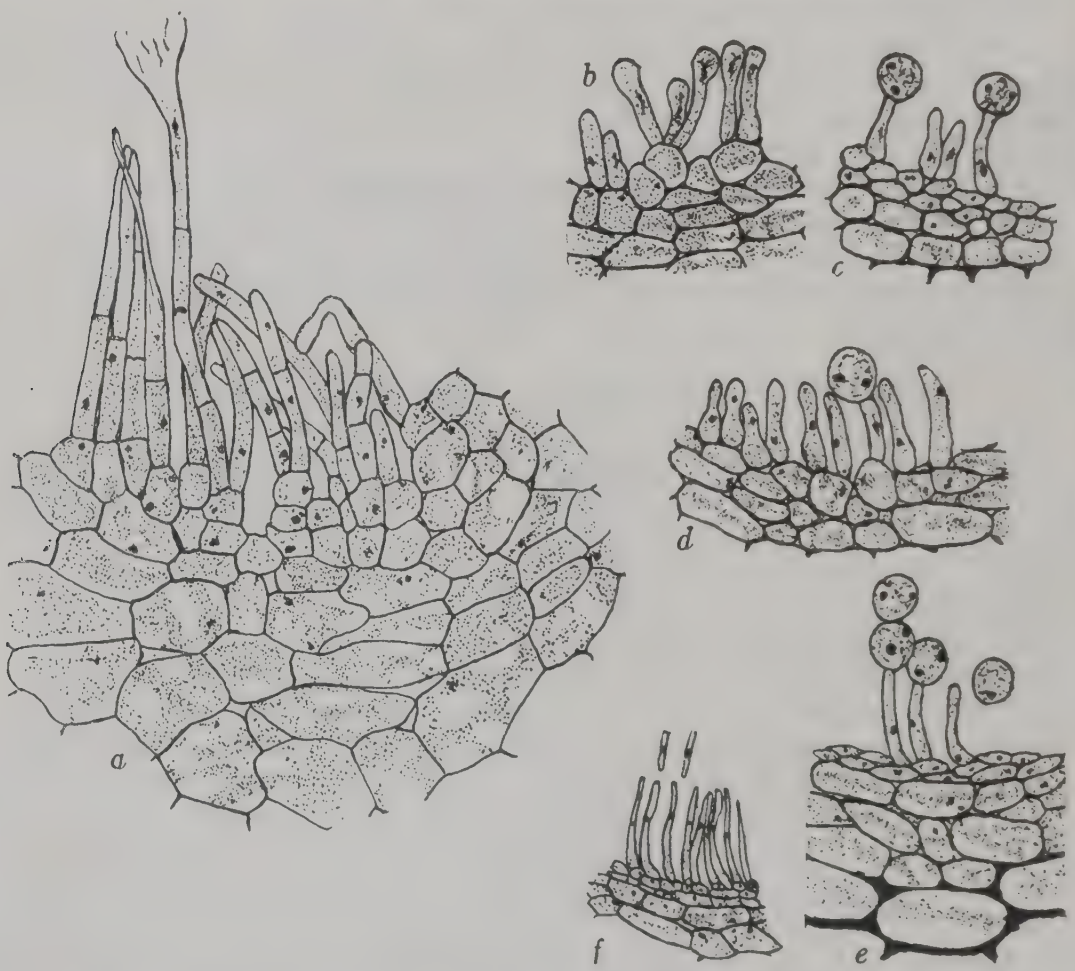

FIG. 1.—Development of sporophores: a, Hyphalike protosporophores growing out from cells of wall which is as yet not definitely organized, showing ends of hyphae undergoing disorganization; b, the first sporophores, sometimes several budding from a single cell, with ends of sporophores swelling in first stages of spore formation; c, sporogenous layer now definitely organized; d, young sporophores, each with a single nucleus; e, sporophores from a mature pycnidium, each spore binucleated; f, spermatiophores from a mature spermogonium.
All drawings made with the aid of a camera lucida Zeiss No. 8 ocular, 3-mm. oil-immersion lens.

very beginning of cavity formation. This may account for the pycnospores sometimes found in spermogonia, to be noted later.

The ostiolar portion has not been developed in the particular pycnidium shown in Plate 1, C; central cavity formation began very early, while the primordium was still little "carbonized" and therefore in a plastic state. Sections often show a large central cavity filled with pieces of the inwardly growing hyphae in all stages of disintegration. New buds to form sporophores arise from the subadjacent cells, which as the result of radial and tangential divisions become much smaller than those which originally occupied this space in the pycnosclerotium. A still older stage is shown

in Plate I, D, where there are no traces of the hyphalike branches; but the big cavity is filled with the stainable disorganized remains, suggesting very strongly that much of the nutrient for spore formation is obtained from within. In more mature pycnidia the central and ostiolar cavities will contain many spores, but no stainable cell remains (Pl. 1, E).

Sporophores are less easily distinguished in mature pycnidia because they are used up more and more as the food supply becomes exhausted in spore formation. This was pointed out by Bauke (*2*). It would be difficult to prove in any case, but it is not improbable that the entire layer of fertile cells bearing the first functioning sporophores may disappear and new sporophores, one by one, grow out from the cells next in line. The cavity certainly increases in size, and the extent of the tissue lining it diminishes. Plate 1, D and E, shows an elliptical cavity in the necklike portion which serves as a temporary storage space for spores.

SPERMOGONIA OF THE FUNGUS

One finds statements regarding the transformation of spermogonia into pycnidia, or vice versa, or of either of these forms into perithecia. No one has given us the story of what happens to the pycnidia covering a particular grape placed out of doors throughout the winter. After a somewhat extended study of this fungus, the writer is still unable to distinguish positively the bodies out of which spermogonia or pycnidia will be formed. He suspects that each of these spore-producing bodies arises from its own peculiar primordium, notwithstanding the fact that it is not at all rare to find pycnospores in spermogonia.

As noted previously (p. 745), the perfect stage of *Phyllostictina carpogena* remains in doubt. Further evidence that it may be a Guignardia is that it has a spermogonium very much like that of *Guignardia bidwellii*.

Spermogonia and pycnidia may be found side by side on the dewberry, and were it not for their contents these fruit bodies could not be distinguished. In size, shape, and wall structure they may be identical. In many spermogonia there is found the same peculiar elliptical ostiolar cavity (Pl. 1, F) so characteristic of pycnidia of this species. The writer has studied much more extensively the formation of the cavity in spermogonia of *Guignardia bidwellii*. The processes appear to be the same in both species.

CAVITY FORMATION IN THE SPERMOGONIUM

Cavity formation begins rather in the apical portion of the primordium of fundamental tissue where by partial disorganization of cells way is made for growth of orientation by which chains of cells become directed toward the apex. This stage, once initiated, is carried on very rapidly. Protospermatiophores which are long and threadlike soon fill the central portion (Pl. 1, F), each hypha (?) consisting of a chain of rodlike cells surrounded by a mucilaginous sheath. These cells, freed from each other, could scarcely be distinguished from spermatia; and it is possible they are the first spermatia formed. Later, when definite spermatiophores (Fig. 1, f.) can be clearly made out, the cavity is devoid of such threadlike aggregations, and very distinct rod-shaped spermatia lie around in the cavity. This suggests that the structures shown in Plate 1, F and G, now break up and disorganize, leaving the mucilaginous products still in the cavity, and that definite spermatia will soon be formed.

There are eight pycnospores in the spermogonium, a section of which is shown in Plate 1, G. There is now no evidence of the origin of these spores, which were probably formed at an early stage in cavity developement; and the cells from which they were derived have now disappeared. The writer has not seen sporophores in those spermogonia of *Guignardia bidwellii*, which contain a few pycnospores.

It has been shown above that spermogonia and pycnidia are formed from bodies similar to what Reddick (*8*) calls "pycnosclerotia." In many species the tissue composing the "gnarled" primordium is not at all sclerotized. Pycnidia of *Schizoarme straminea* Shear, for example, arise out of a tissue that can not be distinguished from a stroma (Pl. 4, A). The very irregular tuberculate and massive forms taken by the "pycnosclerotia" of *Phyllostictina carpogena* in cultures on agar very frequently become arrested at this point, but so many have been found in the process of cavity and spore formation as to suggest that these structures are fundamentally sclerotized stromata, out of which, or in connection with which, fruit bodies are formed. Pycnosclerotia then, if we may put it that way, are not designed to be resting stages in the life cycle, not necessarily structures into which the fungus is forced (even if frequently compelled to remain there) by unfavorable environmental conditions. They are the result of a regular and natural course of events by which certain quantities of food and fungous tissue are brought together in preparation for spore formation. Having seen that in this Phyllostictina there is little actual crushing of tissue such as was thought by Bauke (*2*) to be the case, the relative importance of the two factors, disorganization and growth inequality, in cavity formation can be judged also by the little average difference in size between the "pycnosclerotia" and mature pycnidia. Allowing for cases such as were noted previously in which cavity formation begins very early, the central cavity in this species is ordinarily, then, largely lysigenetic. We may next turn to a pycnidium of an entirely different type.

ORIGIN OF THE CENTRAL CAVITY IN THE PYCNIDIUM OF THE STRAWBERRY-ROT FUNGUS

Associated also with the rots of dewberries and strawberries is the fungus, *Sclerotiopsis concava* (Desm.) Shear and Dodge, and its conidial stage is very common on other plants (*12*). The pycnidium is large, shield shaped, and without ostiole. The big spore cavity is surrounded by a heavy brown wall several cells in thickness and at maturity is densely packed with spores (*12*, Pl. 5, Fig. 17).

The intermediate stages in development are best studied from sections of material grown on strawberries or on infected leaves placed in damp chambers. In the younger primordia, hyphae invade the epidermal cells, bursting through the side walls and lifting the cuticularized layers, forming a broad, compact, mound-shaped tissue (Pl. 3, A), most of the outer portion of which consists of hyphae spreading outward from a broad base (Pl. 3, B). The cells at the ends of these hyphae after a succession of divisions become elongated peripherally. Now along a line 5 or 10 cells from the outside border a narrow zone of degeneration can be distinguished; cells are losing their contents and elongating as though under strain (Pl. 3, C). This disorganization occurs in intercalary cells shown at the right in the figure. The line of rupture is not necessarily so evenly curved that it follows the contour of the outer wall;

it is more often very irregular, dipping down here and there rather deeply (Pl. 3, D). Superficially the pictures presented certainly suggest that the rupture is caused by forces from within, either that the lower two-thirds of tissue has shrunken while the outer third designed as the future wall has remained rigid or that the latter part has been raised bodily by addition of new cells at the ends of the arch, the rest of the tissue remaining dormant. But the early stages of these large, dome-shaped pycnidia develop in nature beneath the tough and tightly stretched cuticle, for example, of canes of *Rubus* spp., where if the rupture were caused in the manner just suggested, there would occur more or less buckling of the outer wall under such unequal strains as must exist. At such a stage (Pl. 3, D) no buckling occurs, which means that the wall must be supported by the pressure from below, furnished by disorganization of the intercalary cells, as indicated. Mature pycnidia on leaves in dry weather are found collapsed, indicating that the thick arched wall in itself is not strong enough to prevent buckling after the pressure has been withdrawn by the shrinkage of the mucilaginous substance within. A concave pycnidium usually indicates that it will be found full of spores. Figures D and E are from a section of the same pycnidium at slightly different planes. Figure E shows somewhat better that the break occurs in radially growing hyphae, but Figure D suggests that disorganization accompanied by the swelling of cells is responsible for the rupture of the tissue. The five or six large bodies mixed in with the tissue just pulling apart (at the center above) are the remains of what just previously were very minute cells of the parallel hyphae and which have now swollen to many times their original size. Many such cells are visible in the section shown at F and also at H, which is still more highly magnified. Above are large masses of small intercalary cells detached from the hyphae which bore them and are now caught between two regions of disorganization. In the section shown at G, the sporophores are fully formed along the entire base; but the mass above these consists only of rows of disorganizing cells, above which is a clear substance, difficult to stain. In this condition the structure might be mistaken for a mature pycnidium nearly filled with spores. Compare this with the section shown at I, which is from a very small pycnidium in which the first spores have just been formed. The spreading, or nearly parallel, rows of cells forming the upper wall are still distinct. The cavity may now enlarge to some extent, and there is little question that growth at the border accounts for some increase in its size. It is noteworthy that there is always more tissue below the line of rupture in a young pycnidium than there is below the true sporophores in a mature structure (Pl. 3, C, D, and I). The cells that are first forced apart in the destruction of tissue and that remain below are homologues of the sporophores, but as noted they provide the material which by swelling forces the wall farther out, incidentally later furnishing food for the development of spores.

It has been shown that the rupture in the tissue is initiated by the disorganization of certain intercalary cells in oriented hyphae and that this occurs not at a period of shrinkage but while expansion is still going on. Pressure is provided by the swelling of the cells torn loose from the wall above or isolated from the vertically growing hyphae below. This force maintains the even curve of the wall as it lifts the resistant portions

of the epidermis above, and marginal growth contributes to the fina enlargement of the cavity. We see, then, that the cavity in *Sclerotiopsi concava* is about equally lysigenetic and schizogenetic, and in this respect represents a type intermediate between *Phyllostictina carpogena*, noted previously, and *Schizoparme straminea*, which will now be considered.

SCHIZOPARME STRAMINEA, A NEW STRAWBERRY DISEASE

Pycnidia of a fungus heretofore unreported on strawberry have been found on this fruit from Norfolk, Va., and in the markets of New York and Washington each spring since 1918. The fungus is not abundant, and it does not cause a destructive rot of the berry. The pycnidia are thickly scattered over the surface bursting through the epidermis at the time the spores are mature (Pl. 5, A). The fungus is distinguished from others on the strawberry by the crown of light-colored tissue which surmounts the pycnidium and surrounds the ostiole. The same species also occurs on dead leaves of strawberry and several other plants. In July, 1920, perithecia were found on strawberry leaves along with these pycnidia, and the connection between the two forms was proved by growth in pure cultures. Pycnidia and perithecia are illustrated in Plates 4, 5 and 6. A formal description of the fungus is to be published by Doctor Shear in an early number of Mycologia.

CULTURE STUDIES

The unicellular pycnospores germinate with one or two germ tubes without showing septation previous to or during germination. On corn-meal or potato agar in Petri dishes the mycelium grows outward unevenly, so that the margin is much scalloped or lobed, (Pl. 5, B). Pycnidia are formed in concentric circles, the first maturing five or six days after inoculation. They are at first colorless and are surrounded by a loose weft of hyphae. Sclerosis begins at the base of the ostiole and spreads around the wall along the outer layer of cells, so that the pycnidium finally becomes nearly black.

Artificial infection of strawberries has been made to obtain material for study. The fungus is able to maintain possession except when species of Rhizopus gain entrance at an early stage.

In Petri-dish cultures there is a tendency toward the grouping of pycnidia, with an especially large one at the center (Pl. 5, D). They are held together by the tissue which crowns each one and which now viewed from above resembles a thin stroma; the fruit bodies are always separate in nature.

The ascospores are small and hyaline and thus are not readily located in poured plates, so that the following method was used to obtain pure cultures. Single ascocarps were crushed out in a drop of water on sterilized slides. The asci still containing spores float out in the water. Small drops of this water were transferred to a 4 per cent corn-meal agar in Petri dishes at marked places. As the drop spread out and was absorbed, single asci were located. The spores germinated in the ascus in a few hours, so that by transferring the entire ascus after germination one was able, very easily, to obtain large numbers of pure ascospore cultures. Over 100 single ascus cultures in test tubes and Petri dishes were made in this way, and in every case characteristic pycnidia were

formed in a few days. No perithecia have been seen in cultures either from ascospores or pycnospores.

FORMATION OF THE CENTRAL CAVITY IN THE PYCNIDIUM

The various stages are best studied in material obtained by artificial infection of strawberry and from cultures on agar media where the early stages are somewhat exaggerated and prolonged, so that the growth story can be followed and timed more easily than from pycnidia obtained on fruit and dead leaves. Sections of the pycnostroma show a rather homogeneous plectenchyma of thin-walled cells without any trace of sclerosis; consequently, as we should expect, the force due to the more rapid growth of the peripheral cells resulting in the rupture of inner cells comes most easily and naturally into play.

When the primordium has attained considerable size, forming a spherical or elliptical mass of tissue 50 to 100 microns in diameter (as it is found on fruit or on leaves in nature it is much less), it has stored in it a quantity of food readily available for the developments which are next to take place. The sporogenous tissue will be located on a dome-shaped structure placed at the bottom of the cavity similar to that of *Coniothyrium diplodiella* described by Istvanffi (7); the curve of the dome will correspond roughly with that of the wall above, with a spore cavity between the two tissues. The region in the plectenchyma where the cavity will first appear can be determined in advance of any line of rupture. There is a preliminary growth and orientation by which rows of vertically placed cells are laid down in a line across the upper central portion of the primordium. The upper cells in these rows stretch out or swell as gelatinization progresses, but the cells below the line of final separation continue to grow upward, forming a palisade-like row of elongated cells (Pl. 4, A). As noted, the cells that first rupture appear to be either swollen longitudinally or stretched. If the rupture were due to the excess growth of cells in the periphery over that of the cells within, this appearance of stretching would be a natural one; but it should be kept in mind that there has been a vertical growth of the hyphae that are being ruptured. The disorganization may thus be due to the thrust of the growing cells into those above, or to enzym action. In any event these elongating cells are the homologues of sporophores, although the ones present at this time will not bear spores. The end cells disorganize and the colloidal remains stretch out as the cavity increases in size (Pl. 5, E). The end cells slough off, new buds put forth, and new potential sporophores arise by division of cells of the enlarging dome of fertile tissue. In material grown on agar there is certaily a large amount of disorganization of tissue of the pycnidial stroma, but in nature this structure is not great, and differentiation of tissues begins very early so that the lysigenetic enlargement of the cavity is less apparent. That there is here a certain amount of cell destruction is evident from the cell remains still clinging to the inner layer of the wall of the mature pycnidium (Pls. 4, J; 6, F). So characteristic is this line of disorganized cells that our fungus was recognized in sections of oak leaves from Italy collected by Professor Massalongo 30 years ago.

DEVELOPMENT OF THE PYCNIDIUM ON STRAWBERRY

On strawberry the pycnidial primordium reaches but little size befor it can be seen in sections that the cells just below the peripheral laye at the top are being oriented vertically (Pl. 4, F). There are at firs not more than a half dozen rows, two or three cells in each row, in th palisade (Pl. 4, G). These appear, as it were, to be thrust against th periphery, flattening out the cells so that one would say that the wa now consists of only two or three layers of cells, disregarding the buffe tissue which is now burrowing through the host above and which take no part in the formation of the wall. The vertically oriented hypha now branch out and their end cells disorganize, the additional pressur due to mucilaginous disorganization tending in all cases to play a stil further part in the organization of the inner-wall cells. These may in tur degenerate or, if not, they may divide tangentially or elongate peripher ally so that finally the completed wall consists of five or six layers o cells which are two or three times as long as thick. Sclerosis is so lon delayed that the pycnidium is capable of continued enlargement.

GROWTH OF THE BUFFER TISSUE AND FORMATION OF THE OSTIOLE

The formation of the ostiole in perithecia of a few species has bee described by Füisting (*1*), De Barry (*3*), Miyabe (*4*), and others, and i has perhaps been assumed that the same principles hold in the develop ment of the ostiole of the pycnidium. In some perithecia there is a dis organization of a strip of tissue leading from the hymenial cavity to th outside. Further enlargement of the ostiole takes place schizogenetically

In nature the pycnidia of *Schizoparme straminea* are deep seated, and the papillate ostiole scarcely reaches the surface. The work of rupturing the epidermis and other host tissues above is performed by a sort of buffer tissue from the outer loose hyphal envelope, the "tissu fendant" of Istvanffi (*7*) or the "Aperturalgewebes" of Füisting (*1*). An opening must be made through the buffer tissue before the spores can be dis charged. It will be shown that the ostiolar opening is formed first through the wall, then passage way through the buffer tissue will be made. The sharp papillate beak results from the upward growth of buds from wall cells.

Soon after the formation of the central cavity has begun, as noted above, the cells at the top of the young pycnidial knot multiply rapidly, burrow into the epidermis, and increase in size by swelling to form a cone-shaped tissue, which is very effective in lifting up, pushing aside, or break ing down the host tissues overlying the pycnidium, after which it appears above the surface as a whitish or cream-colored hood (Pl. 5, E), similar to the "tissu fendant" surmounting the pycnidium of *Coniothyrium diplo diella*. The perforation of the buffer tissue and the disorganization of the wall of the pycnidium in the preliminary stages of ostiole formation are two distinct processes, yet they are both closely associated and neces sary in the development of the complete apparatus of spore discharge.

The construction of the ostiole begins at a point in the incipient inner-wall tissue above. A few cells next to the central cavity now forming (Pl. 4, B) begin to disorganize and by swelling provide a small space into which adjacent wall cells bud, turning upward (Pl. 4, E). In the mean time the buffer tissue has developed and performed its function. The disorganization of the wall cells progresses so that the ostiolar opening is

made first, after which disorganization spreads in several directions, and it is the outermost cells of the buffer tissue that are last broken down (Pl. 4, D). The swollen cells of which it is composed contain very little cytoplasm; but there are certain regions in which they show signs of degeneration, accompanied by still further swelling, so that the hood becomes split or ruptured in a very interesting fashion, the lines of cleavage radiating from the point below at which the beak of the ostiole is being formed (Pl. 5, D).

The cells of the buffer tissue still possess the power of growing, for as soon as a cavity has been formed in it by disorganization small hyphal branches bud out (Pl. 4, E). These branches are very soon entirely reabsorbed.

By the time a passage way has been opened through the buffer tissue, other short hyphae have begun to appear from the wall cells, converging to form the beak of the ostiole. The cells lining the ostiole are certainly the homologues of periphyses, but in this case they usually disorganize so that in a fully mature fruit body (Pl. 6, B) little trace of them remains. In the meantime the central cavity has become greatly enlarged, and the remnants of broken-down cells may be seen clinging to the inner wall, as noted above (Pl. 4, J). It is interesting to find that in the ascocarp of this fungus (Pl. 5, F–H) the host tissues are broken open by the same sort of buffer apparatus. This is disorganized in the same fashion and penetrated by the ostiole in like manner; the periphyses are, however, more permanent (Pl. 5, J). In the pycnidial cavity there is much more disorganization of tissue when the fungus is grown on agar media where the absence of confining host tissues may be an additional reason for the delay in the differentiation processes.

Other stages in the life history of *Schizoparme straminea* are illustrated in Plate 5. The habit of growth of pycnidia on the fruit of strawberry is brought out in Figure A, which is somewhat magnified. The buffer tissue which has broken through the epidermis of the host is the only part of the pycnidia visible. On agar media in Petri dishes the mycelium appears to break up into fan-shaped lobes, and the pycnidia form more or less in concentric circles (Fig. B). Surface views of pycnidia on agar such as are shown in Figures C and D bring out very strikingly the manner in which the buffer tissue splits open, frequently in the form of a cross.

That the buffer tissue is composed of cells originating from the upper portion of the outer wall of a pycnidium is clear from the sections shown in Figure E. It is often difficult to distinguish perithecia from pycnidia by the appearance of the buffer tissue exposed, although the ascocarps are frequently much larger than pycnidia. Figure F shows a number of perithecia as they appeared on a much decayed leaf of *Rosa* sp., bringing out well the manner of splitting the buffer tissue, which is so characteristic of the tissue above pycnidia also (Figs. C and D). Mature ascocarps collapse when dry. Sections of a young perithecium through the papillate ostiolar portion (Figs. G and H) show that the buffer tissue evidently functions in rupturing the host tissue, although it soon dries up and falls off after bursting through the epidermis (Fig. I). It is clear from Figures I and J that there are no paraphyses between the asci, although falcate periphyses line the ostiolar cavity. A mature ascocarp with fragments of buffer tissue still adhering is shown in Figure K.

We have in this species a case where the sexual and the asexual fruit bodies are very similar in several respects. The walls of both structures are composed of similar tissue, which is carbonized only at maturity. The ostiole in each form is prominent, being more or less papillate. The straw-colored buffer tissue is always present in both forms, splitting open in a characteristic fashion. The pseudoparenchymatous tissue at the center of the ascocarp breaks down, thus forming a central cavity. One need not confuse the two forms, since the perithecia react to the triple stain in a manner quite different from pycnidia.

The very striking morphological parallelism shown in pycnidia and perithecia of this species raises again the question as to what extent this really occurs among other Ascomycetes, or how much dependence can be placed on such features in the search for other spore forms in the life cycle of a given fungus. Orton (*9*) called attention especially to various cases of similarity between pycnospores or conidia and the ascospores of certain species. While many examples where there is no such resemblance between the types of spores may be pointed out, it must be admitted that it would be a very natural morphological expression of the inherent physiological or metabolic activities, based upon the constitution of the germ plasm. A feature worked out in the course of time for one spore form must of course have a basis in the apparatus of inheritance. It is surprising that these morphological parallelisms are not more frequent in the Ascomycetes, until one considers the particular environmental conditions under which each type of spore or sporocarp performs its work. The spores of *Hainesia lythri* (Desm.) v. Höhnel and *Sclerotiopsis concava* (*12*) are identical, but the sporocarps bearing them have little in common, because the latter form has taken on the overwintering function. On the other hand, the discocarp stage, Pezizella, resembles the Hainesia.

ABNORMAL ORIENTATION OF SPOROGENOUS TISSUE

It is well known that in cultures of some forms of Ascomycetes it is possible, by changing the direction of illumination, to cause pycnidia or ascocarps to be formed in an oblique or an inverted position. In such cases the structure is inverted or changed as a whole. By suitable orientation of cultures of *Schizoparme straminea* with respect to light, gravity, and aeration the fungus can be induced to build sporogenous tissue at the top of the pycnidial cavity so that the sporophores and spores point downward as they are formed, while the buffer tissue and the ostiole develop in the normal fashion, pointing upward. Figure C in Plate 4 shows a stage in the development of the pycnidium just preceding spore formation. The buffer tissue is not quite full grown, but other sections in the series show that an ostiole is just being formed through this tissue at one side of the sporogenous pulvinus so that, had this pycnidium been allowed to mature and then been cut in a plane at right angles to the one shown here, the relative positions of the sporogenous tissue, buffer apparatus, and ostiole, would be the same as shown in Pl. 6, C. In case the fertile tissue had been directly at the center above, there would have been two ostioles formed (Pl. 6, D); this is in no way associated with two locules in the early stages. What bearing can these curious inversions of the sporogenous tissue have on the question of the methods of cavity formation? This is a complicated situation where we have a central cavity formed, sporogenous tissue delimited,

buffer tissue developed through which a passageway is opened, a wall organized, an ostiolar opening through the wall portions broken down, and a beak developed. The fungus must be able to organize a number of varied localized changes and growth processes harmoniously interacting in such a way as to lead to the construction of a fruit body with certain constant diagnostic morphological features. Such a structure, the cavities or openings in which are formed purely schizogenetically, is difficult to conceive.

As *Schizoparme straminea* is found in nature there is nothing to suggest the presence of a stroma, except perhaps the buffer tissue ramifying through the host tissues and bursting forth, crowning the pycnidium. As explained, this tissue is formed after the knot of undifferentiated primordial hyphae has reached considerable size. Only one pycnidium grows from each knot of hyphae. On agar, however, several pycnida may arise from one of these masses of plectenchyma in such a way as to suggest that the line separating such pseduoparenchymatous primordia from true stromata is not to be too sharply drawn. Again, cavity formation may be started at several different points, followed by a merging of the locules, so that the cavity is at first much lobed or irregular; it finally becomes rounded out by further disorganization readjustments. A multilocular pycnidium would be a very simple step in the evolution from such a type.

As noted previously, there is only a relatively small amount of destruction of tissue during the formation of the central cavity. The ring lining the cavity (Pl. 4, J; 6, F) represents practically all of such disorganization that occurs as the fungus grows on species of Rhus or Quercus. Ostiole formation is usually not begun until the central cavity is distinguishable (Pl. 4, B and E), but no set rule seems to be followed. Ordinarily the first spores are matured before the ostiole is completed. Figure B shows a section in which the position of the ostiole is already determined by the disorganization of cells. The section (E) shows the proliferation of cells of the wall of the pycnidium and the enlargement and disorganization of cells of the buffer tissue.

Füisting (*1*) pointed out how difficult it was to find the early stages of perithecia which he was studying because there is nothing to indicate where they are forming until the Aperturalgewebes bursts through the host tissue, and by that time the perithecium is nearly mature. The same difficulty is encountered in connection with both perithecia and pycnidia of *Schizorparme straminea* as the buffer tissue is not visible until after the first steps in cavity formation have been taken.

DISCUSSION

Partly developed pycnidia of the type found in *Phyllostictina carpogena* have been considered by some authors to be merely young or undeveloped perithecia, especially when they are found in cultures. Some fungi develop vegetatively very well under artificial conditions and even reach the first stage in pycnidium formation to the extent of laying down the plectenchymatous primoridum, yet lack the particular stimulus which sets in motion the metabolic activities initiating the intermediate stages of growth which are fundamentally more complicated than the mere piling up of food in the form of undifferentiated tissue of the primordium.

Very often cell destruction is accompanied with much swelling, which may not be without effect in stimulating further action. In *Schizoparme*

straminea there is a characteristic intercalary growth of orientation before any disorganization sets in. When a weak point is once established and an initial lysigenetic cavity formed, then growth readjustments can come into play, as the results of which the structure may increase in size by the elongation of elements already present at the end of the first period of growth. Intercalary growth in the wall is stimulated, and new cells may be added to the exterior.

Bauke found that in the pycnidium of Cucurbitaria the primordium was often as large as the mature fruit body; therefore, to account for the cavity he supposed that the sporiferous layer was pressed outwardly against the intervening parenchyma so that its cells were crushed. The ultimate effect of pressure due to disorganization is certainly a factor to be recognized, but it is very doubtful whether the cells referred to are crushed to the extent claimed. Those just outside the sporiferous layer are frequently small and flat, due to division, and are not the crushed remains of the large cells once at this point in the primordium. This is quite evident, for a study of Bauke (*2, fig. 12 and 13 of Pl. II*), will show that the cells in the tissue said to be crushed must have undergone division in the process, for the ratio of the number of cells in the periphery of the primordium shown in his Figure 12 to the number in the layer beneath the sporiferious layer is as 3 to 2, while in the mature pycnidium shown in his Figure 13, this ratio is as 2 to 3. Bauke (*2*) and Baccarini (*5, 6*) both state that in old pycnidia sporophores disappear entirely, and many pycnidia have been formally described by authors as without sporophores. The writer has shown that the first hyphae to grow into the initial cavity are not necessarily spore bearing, though they are homologues of sporophores. In *Phyllostictina carpogena* the number of sporophore mother cells increases by multiplication of cells, by radial division, or by budding, while in *Sclerotiopsis concava* and *Schizoparme straminea* the sporophores increase by branching or budding of cells in the dome-shaped fertile tissue. Every cell in the thin-walled central region is potentially sporogenous. Sporophores are often sufficiently marked to be diagnostic, but that they are constant or permanent structures is not borne out by the facts learned from a study of these intermediate stages. That the sporogenous tissue by nature is fundamentally different from every other in the primordium and that, once set apart in the early ontogeny, it remains a permanent, unchanging reproductive tissue is an idea probably carried over from zoology.

Whether the contour of the sporogenous tissue is at all determined by the particular method of cavity formation may be very doubtful, but it so happens that the fertile tissue of the Phyllostictina blackrot of dewberry is strongly concave in outline; in *Sclerotiopsis concava* the incipient or potential spore tissue is convex, but by further disorganization and growth readjustments it soon becomes flattened out so that it forms an even layer extending across the base of the broad flat pycnidium; in *Schizoparme straminea* the fertile layer is sharply convex. It has been shown that in the first species the central cavity is largely lysigenetic. The cavity contains now a mass of predigested food readily available for spore formation, and it should be borne in mind that the fungus provides a large amount of food for this work when the fundamental tissue is developed so that in this pycnidium it is not necessary that more should be drawn from the vegetative hyphæ. In *Sclerotiopsis concava* the central cavity is perhaps about equally lysigenetic and schizogenetic. It is formed in much the same way as it is in the deep-seated

aecidia of the rusts [Peridermium, Adams (*11*)], where the gametes are intercalary cells below in a series of sister cells which are becoming disorganized or have been sacrificed in order to make space and to furnish food for spore production. In *Schizoparme straminea* the cavity is largely schizogenetic, although in artificial cultures on agar there is a large amount of cell destruction.

Some information has been obtained showing that the same factors, disorganization and inequalities in growth, are operative in ostiole formation. In view of the fact that the natural relative positions of the sporogenous tissue and the ostiole can be altered in a very striking manner in cultures of *Schizoparme straminea* it is much less difficult to conceive of a localized weakness in the pycnostroma determined by metabolic changes leading to disorganization which initiates cavity formation than it is to understand how the two different regions of rupture initiating the central cavity and the ostiole can be provided for so long in advance in a purely mechanical way.

In *Phyllostictina carpogena* there is usually a secondary cavity formed in the necklike extension of the immature pycnidium. This is an elliptical cavity which is finally continued downward into the pycnidium and thus becomes a part of the ostiole. Not infrequently it becomes from the first a superficial accessory pycnidial cavity lined with sporophores which cut off spores. These sporophores soon become gelatinized and an opening is formed connecting the two cavities. Sometimes the ostiolar portion is rather small and the cavity is lined with short periphyses, showing in a very striking manner that sporophores and periphyses of this species are homologous structures, or that periphyses may on occasion cut off spores.

The picture of *Schizoparme straminea* (Pl. 4, E) shows that no sooner has an opening been effected in the pycnidial wall by means of disorganization than the cells about the opening bud out or proliferate rapidly, growing into the space and turning upward, after which some of the cells at the ends of these new hyphæ in their turn become disorganized. What is it that stimulates the comparatively old wall cells to such a rapid renewal of growth? We see that there must be a change in pressure, and a new supply of food readily assimilable is furnished by the destruction of cells next to them. Even the swollen cells of the buffer tissue seemingly almost devoid of cytoplasm are still able to grow once the adjacent cells furnish a little food through disorganization.

SUMMARY

The intermediate stages in the development of the pycnidia of three species of fungi associated with fruit rots have been studied, and in each case it has been shown that the initial stage in cavity formation consists in the disorganization of cells in a certain region, accompanied by a swelling of the cell remains. The pressure thus resulting tends to enlarge the cavity, maintain a symmetrical contour, and contribute further toward cell destruction or change of form. Further enlargement of the cavity may be effected by the growth of elements already present or by the addition of new tissue.

In *Phyllostictina carpogena* the cavity is formed lysigenetically for the most part, although occasionally excessive peripheral growth plays some part in the process, especially if cavity formation begins early or before sclerosis has become far advanced.

In *Sclerotiopsis concava* the central cavity is about equally lysigenetic and schizogenetic. The swelling of certain cells of the more or less parallel hyphae composing the primordium causes a rupture along irregular lines, extending across the pycnidium, above and below, thus cutting out a strip of tissue which soon completely disorganizes. The cavity is further enlarged by the addition of new tissue at the border of the pycnidium. There is no ostiole in this pycnidium; spore discharge is effected through the bursting of the wall due to the swelling resulting from inbibition and the partial or complete destruction of certain spores at the center of the mass filling the pycnidium.

The central cavity in *Schizoparme straminea*, as it grows on strawberry, is largely schizogenetic. A ring of disorganized tissue usually lines the cavity clinging to the wall. A buffer tissue through which a passageway is opened by disorganization develops soon after the primordium has been laid down. The ostiolar cavity is formed as the result of the breaking down of the cells of the wall in the early stages; the adjacent cells bud out and hyphae grow upward, converging to form the papillate ostiolar structure. In the meantime by the rapid destruction of the buffer tissue along lines radiating from a point above the ostiole this caplike structure is split open in a characteristic fashion.

The first hyphae to grow into the central cavity as it is forming are the homologues of sporophores, but they do not necessarily produce spores, since those first appearing usually disorganize, furnishing substances which by swelling may play some part in shaping the tissues of the wall.

Food for spore production is found in the parenchyma at the center of the immature fruit body in the first two forms. The pycnidium of the third species remains plastic for a long time, so that additional food may be easily obtained from the substratum upon which the fungus is growing.

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PLATE 1

Phyllostictina carpogena Shear

A.—Section of a "pycnosclerotium"; outer cells darkly colored; central cells thin-walled.

B.—Similar structure, a little older. An open space nas been formed, and into this space the next surrounding cells have grown.

C.—Further increase in size of the central cavity. Disorganizing cell remains lie about in the cavity.

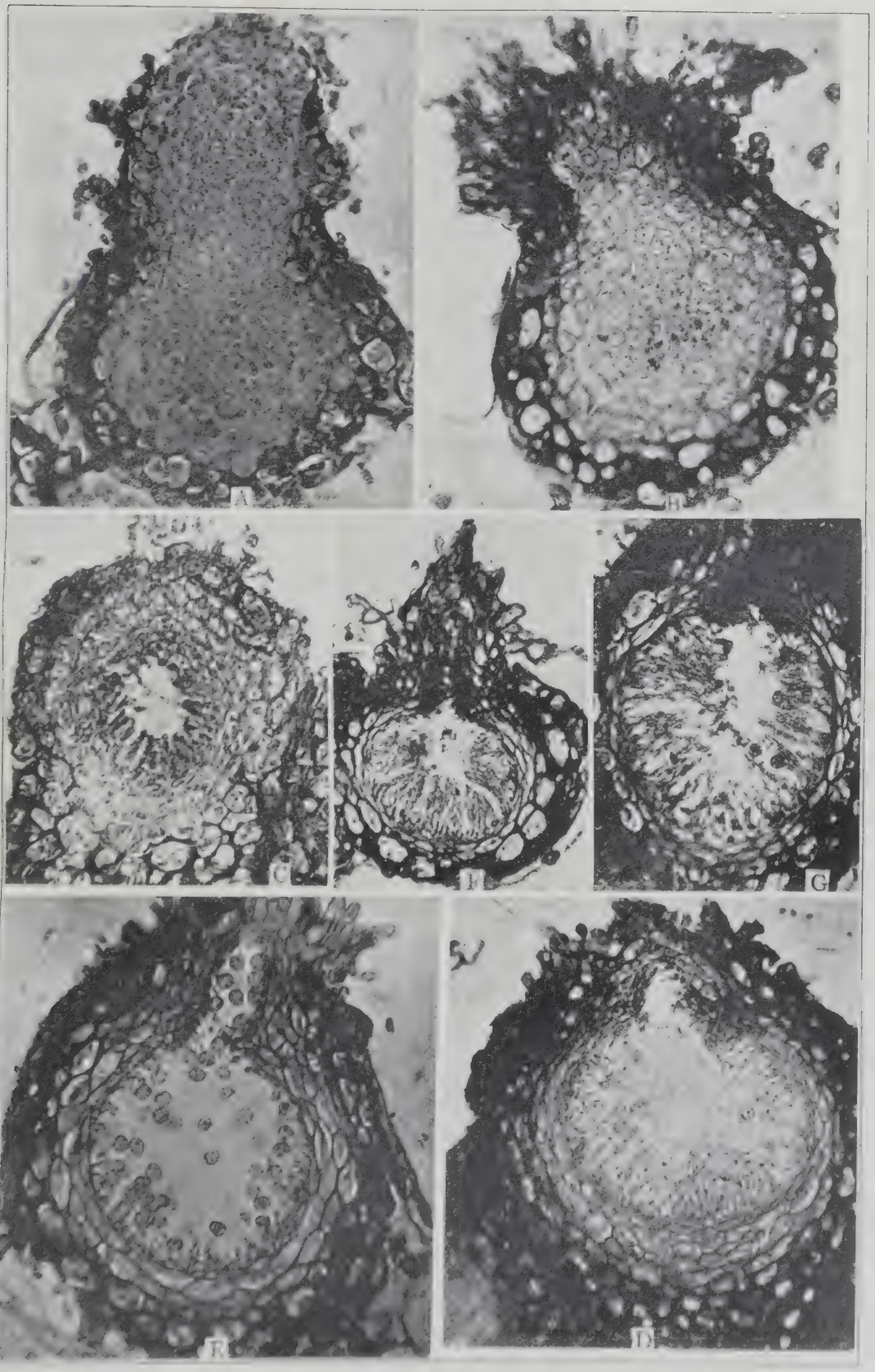
D.—Fully organized pycnidium.

E.—Mature pycnidium, showing a small elliptical ostiolar cavity.

F.—A spermogonium, showing fine, threadlike outgrowths from the inner-wall cells. They have usually disappeared by the time the first distinct spermatia are formed.

G.—An older spermogonium; spermatiophores clearly delimited; pycnospores in the cavity.

Figures B, C, D, E, and G were photographed with a 3-mm. oil-immersion lens; Figures A and F with a 4-mm. dry lens.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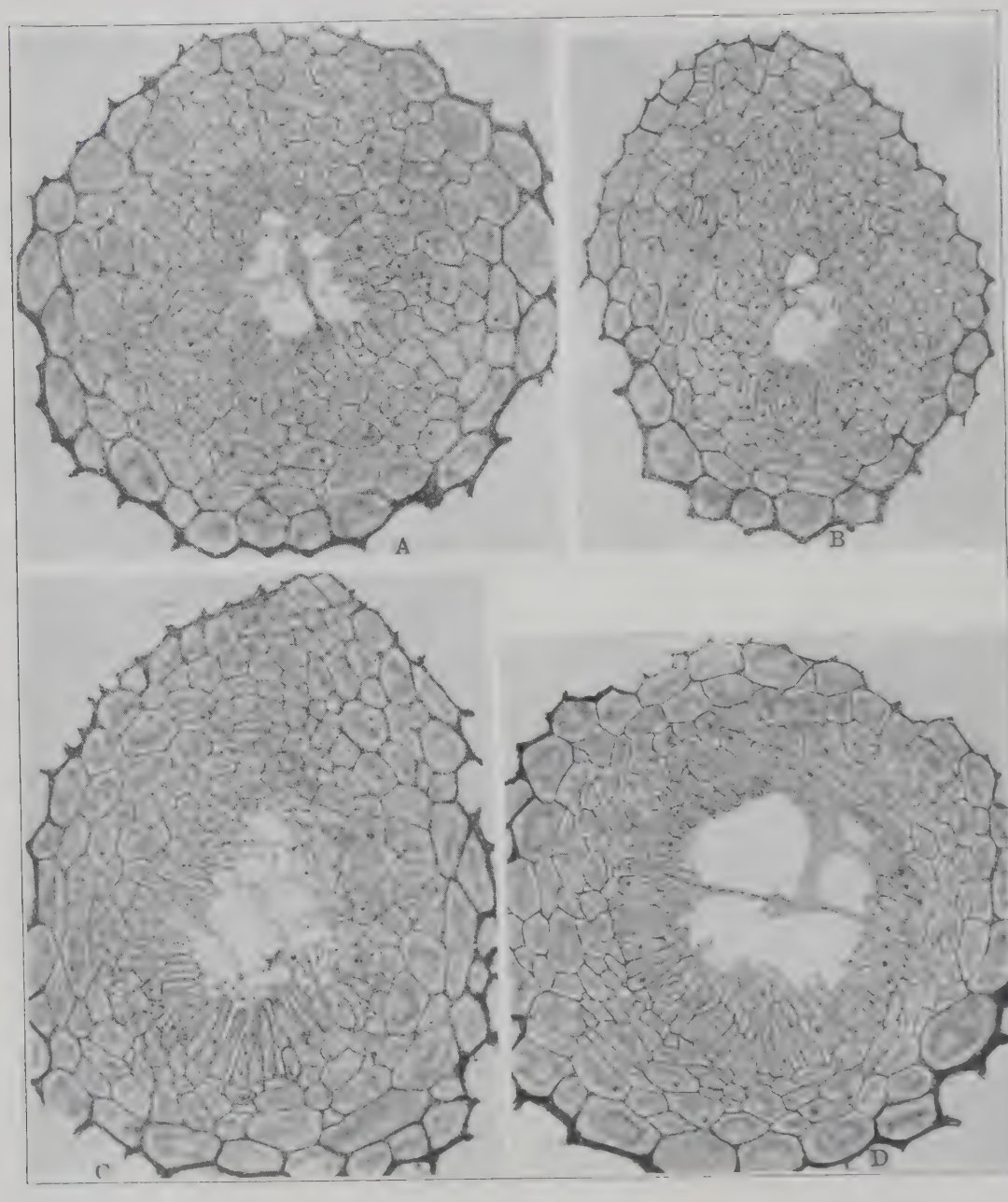
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PLATE 2

Phyllostictina carpogena Shear

A.—Primordium in which a few cells at the center have undergone disorganization; mucilaginous remains of other cells still visible; cells surrounding lower portion of the central cavity beginning to grow inward; two or three layers of "carbonized" tissue surrounding the primordium not shown.

B.—Primordium of a small pycnidium; cavity formation progressing; protosporophores growing inward; "carbonized" portions of outer wall not shown.

C.—Similar to the preceding, but protosporophores more definite.

D.—Layer of cells lining the cavity composed of protosporophores; colloidal cell remains still present in the cavity; inner wall of pycnidium becoming organized; cell divisions in various planes account for the increase in the number of cells.

Figures were drawn with the aid of camera lucida, Zeiss No. 8, ocular, 3-mm. oil-immersion lens.

PLATE 3

Sclerotiopsis concava (Desm.) Shear and Dodge

A.—Mound-shaped mass of hyphae beneath the cuticle; radial growth not evident because this is a somewhat oblique section of the primordium.

B.—Section of a primordium at a slightly later stage, showing plainly hyphae spreading outward from a broad base. The short terminal cells by slight lateral extension take part in the organization of the outer wall.

C.—Still older stage when the line of rupture is well marked. At the right can be seen a small region where the radially growing cell chains have not been ruptured. The break occurs first by the destruction of one or more of the cells of these radially oriented hyphae. The parallel arrangement of hyphal branches below the line of rupture is not the result of an upward growth after the rupture has occurred.

D.—Section of a still older pycnidium, showing quite distinctly five of six swollen cells (center) and other masses of stainable degeneration products in the cavity at the right.

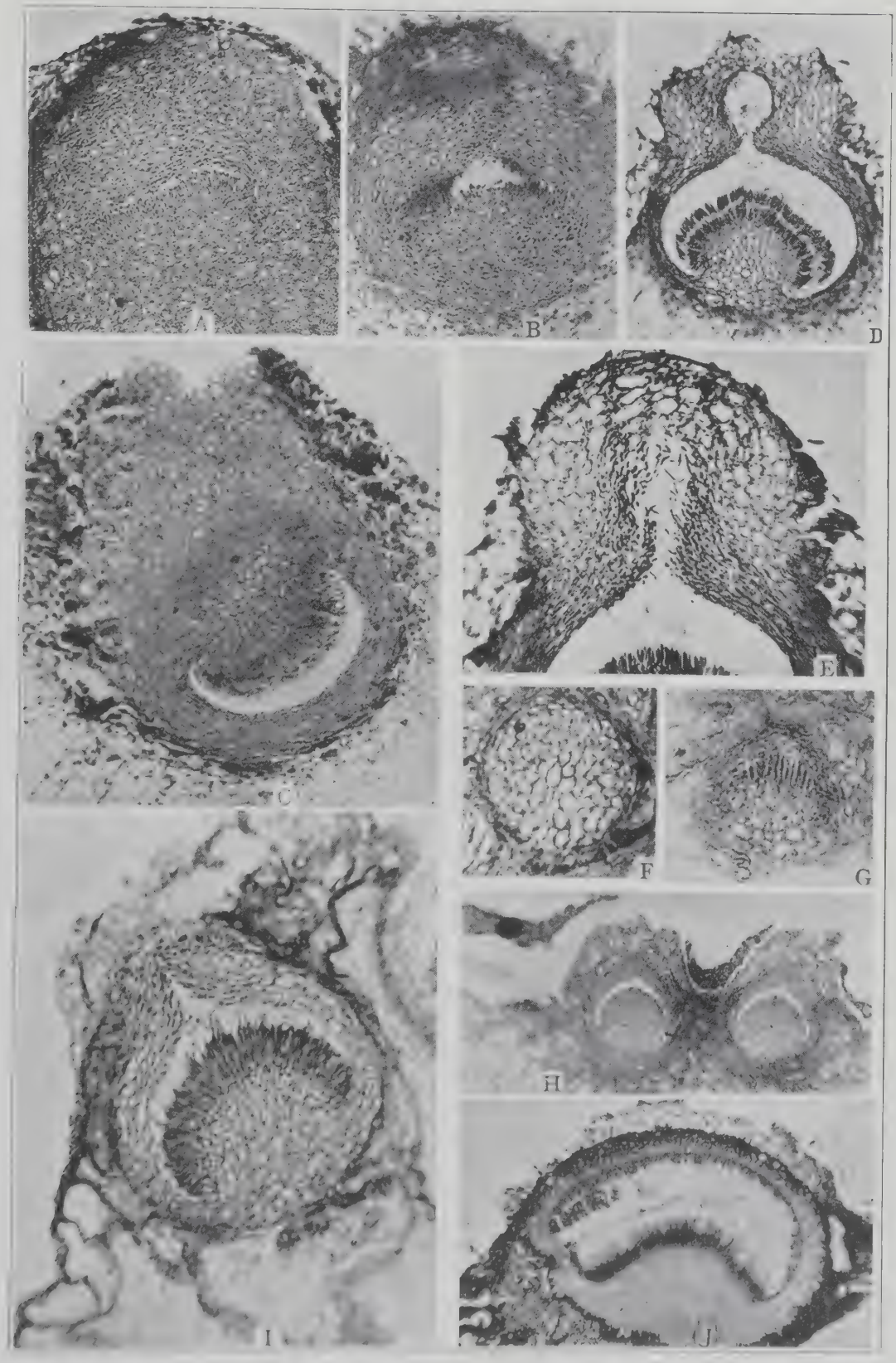
E.—Same section as the preceding except at a slightly deeper focus, showing better how the line of rupture cuts across the radially growing hyphae; degenerating cells at the right.

F.—An older pycnidium, showing many swollen cells.

G.—A pycnidium found on leaf of dewberry; cavity partly filled with masses of detached hyphal cells which originally made up the bulk of the young pycnidial "knot."

H.—More highly magnified picture of a portion of the section shown in F. The very small cells above the large ones are detached portions of the radially placed hyphae, portions of which may become the future sporophores.

I.—Section of a small pycnidium still covered by the tightly stretched cuticle. The first permanent spores formed are still attached to sporophores. There is very little of stainable cell remains in the cavity. The radial arrangement of the cells which make up the wall of the pycnidium can still be plainly recognized.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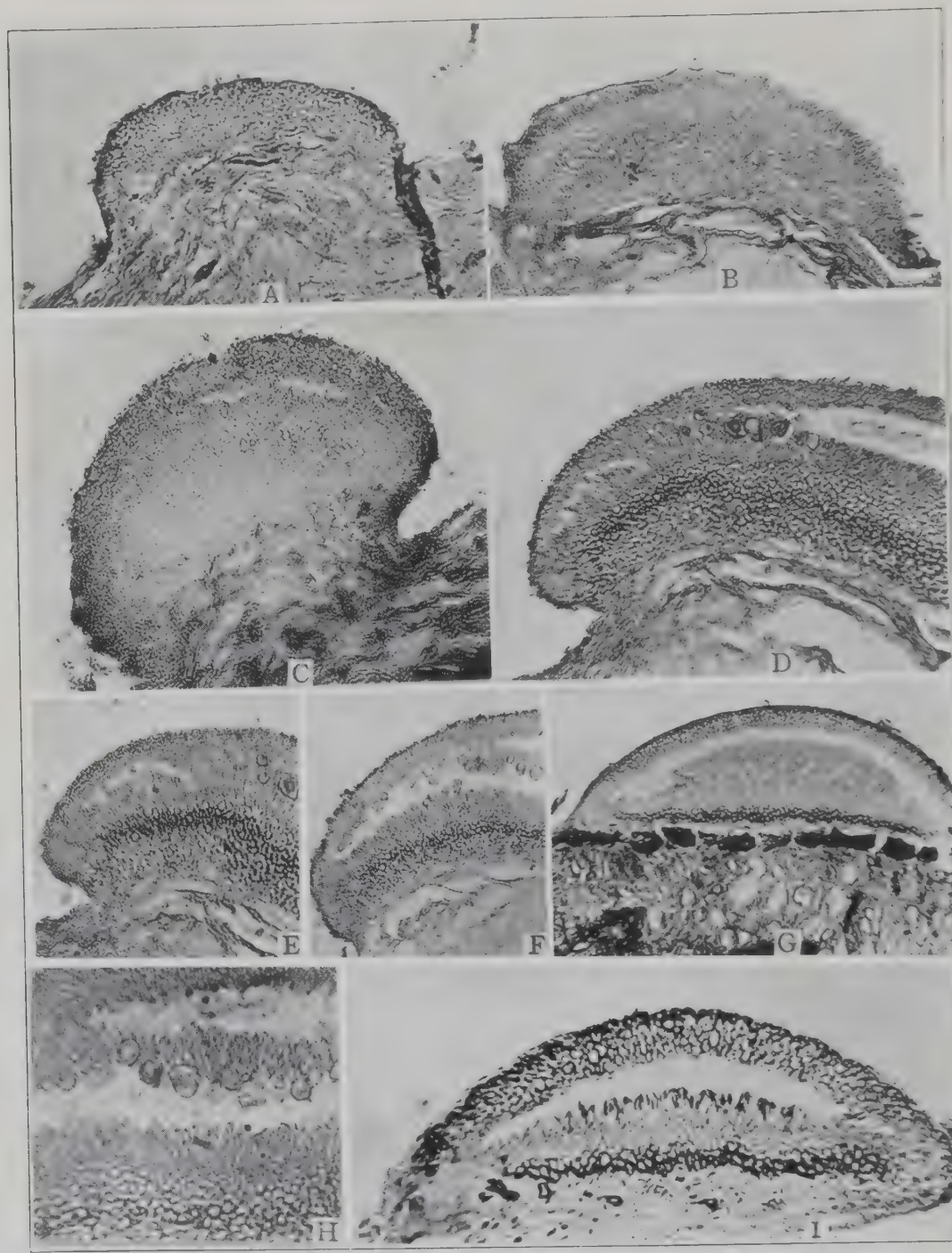
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PLATE 4[a]

Schizoparme straminea Shear

A.—Section at an early stage in the growth of a pycnidium grown on agar; cells above the line of vertically oriented hyphae disorganized, thus determining the line of rupture; no buffer tissue yet formed.

B.—A similar section at a slightly older stage; buffer tissue partly developed; location of the ostiole already determined by some disorganization of tissue; line of splitting below still being extended in both directions.

C.—Section of pycnidium grown on agar under conditions which induce the inversion of the sporogenous tissue; buffer tissue erect and normal.

D.—Section showing, like succeeding one, that the opening or rupture of the buffer tissue is caused by disorganization and not by a tearing apart due to inequalities in growth.

E.—Disorganization of cells of the hood above and proliferation of cells bordering the cavity; buds growing into the space opened by disorganization.

F.—Young pycnidial primordium on strawberry; large inner cells more or less oriented vertically. Above the center these cells are sending out short hyphal branches which are pushing into disorganizing cells in this region.

G.—Similar to the preceding; protosporophores very distinct.

H.—Section of two young pycnidia grown on strawberry. The buffer apparatus has broken through the host tissue above.

I.—Section of a pycnidium similar to the preceding; gelatinizing ends of the incipient sporophores clearly visible.

J.—Section of a pycnidium found on leaves of sumac; remains of disorganized cells lining the inner wall still attached.

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[a] Figures A, B, and H are from photographs by Marguerite Ickis.

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PLATE 5[b]

A.—Pycnidia on a strawberry; only buffer tissue visible.

B.—Characteristic growth of mycelium on agar; pycnidia at first formed in concentric circles.

C.—Pycnidia from similar cultures more highly magnified, showing manner in which buffer tissue splits open frequently in the form of a cross.

D.—In older cultures many pycnidia cluster about the large one first formed at the center.

E.—Buffer tissue seen to be composed of cells originating from the upper portion of the outer wall of the pycnidium.

F.—Large perithecia from a leaf of *Rosa*, sp. They show the same peculiar splitting of the buffer tissue. Mature ascocarps always collapse when dry.

G.—Upper portion of a young perithecium, showing buffer tissue and conical ostiole; opening not yet completed.

H.—Similar to the preceding; opening in the ostiole nearly completed.

I.—Buffer tissue has dried up and broken away. Ascospores just forming; no paraphyses are ever present.

J.—Shows the falcate periphyses lining the ostiole—two branches from each cell.

K.—Mature ascocarp; remains of buffer tissue still present.

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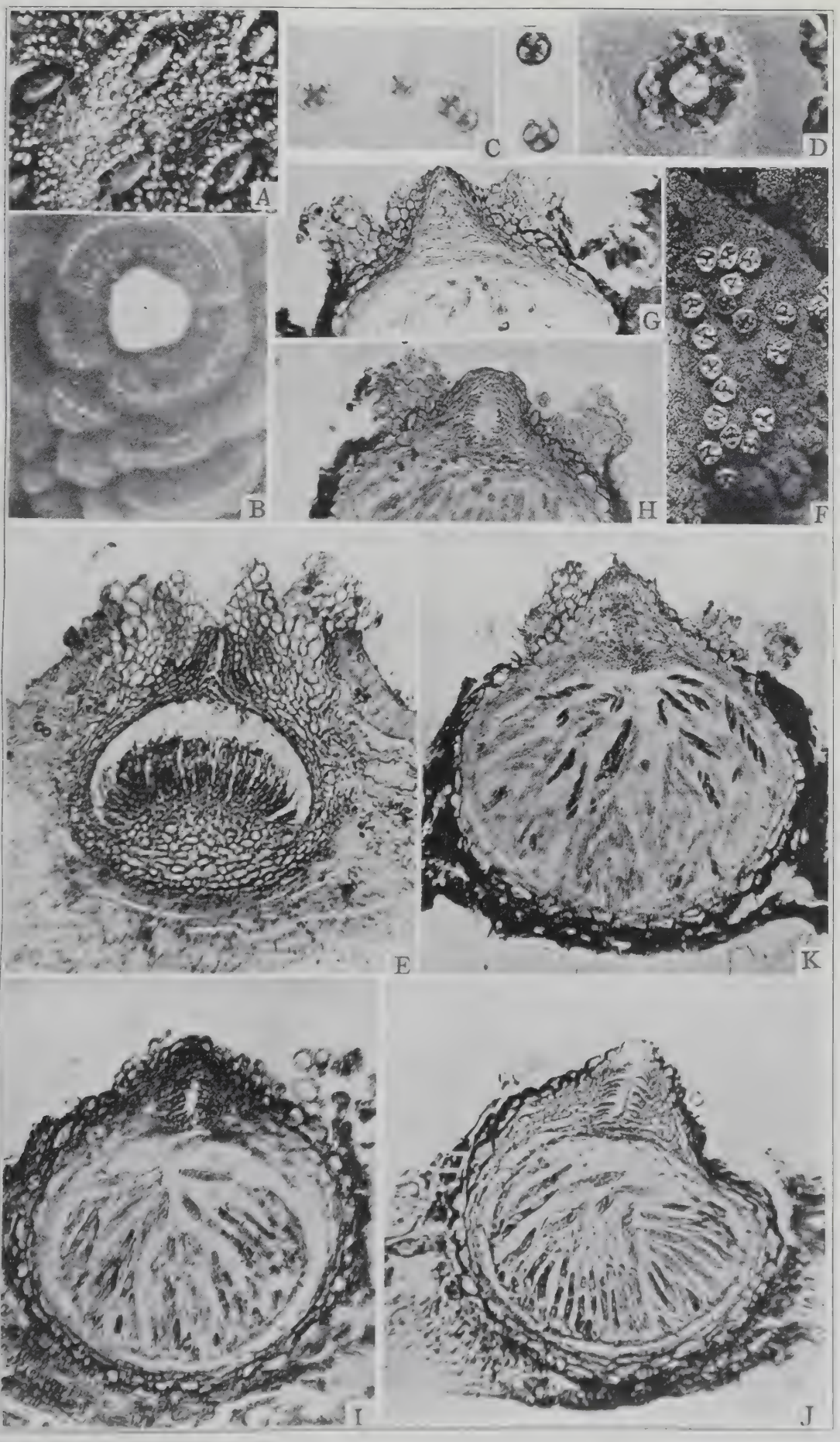
[b] Figures B and C are from photographs by Marguerite Ickis.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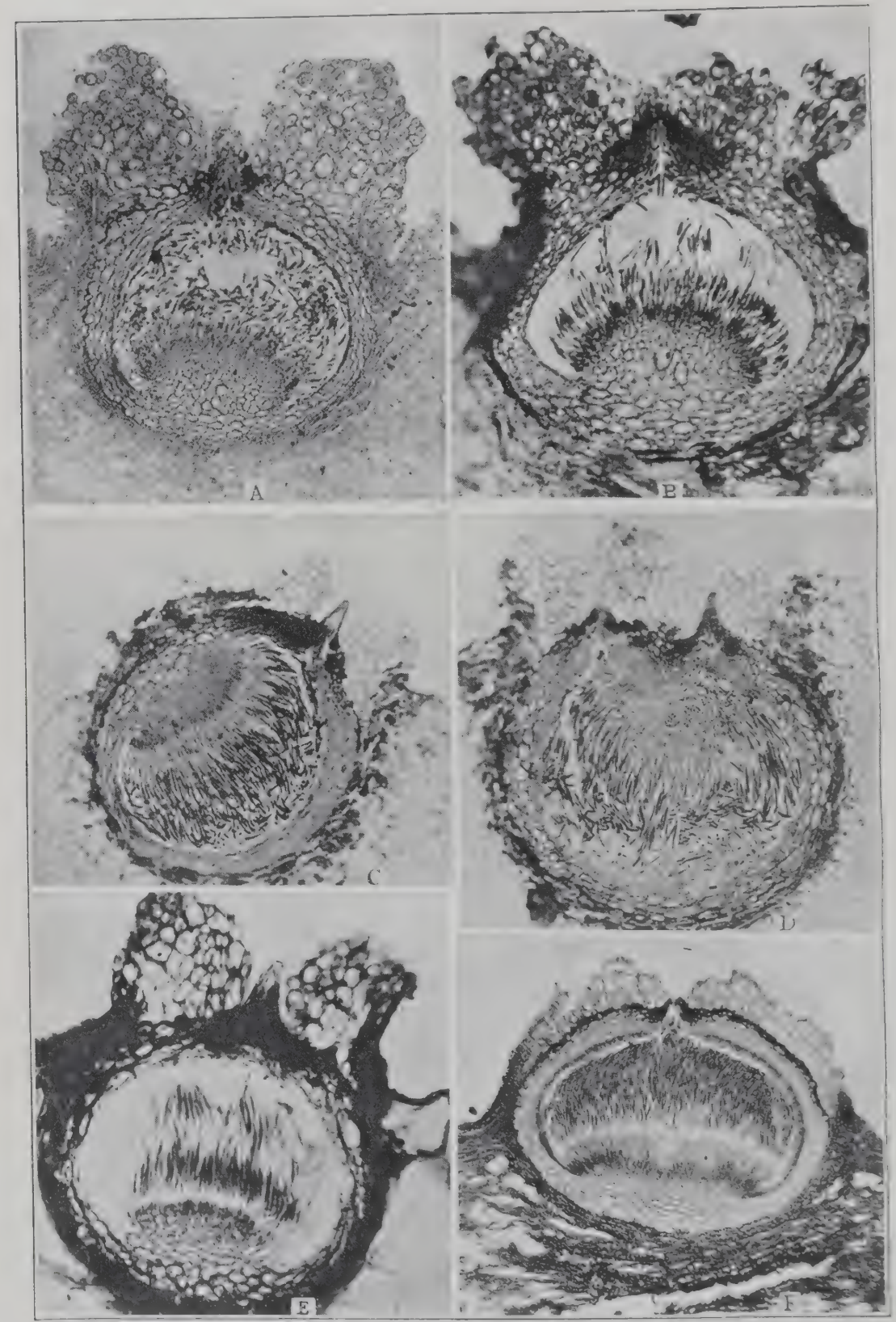
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PLATE 6[c]

Schizoparme straminea Shear

A.—Mature pycnidium grown on strawberry; buffer tissue well-developed; walls of cells surrounding the ostiole thickened and blackened; layer of stainable cell remains clinging to inner wall; sporogenous tissue below.

B.—Black conical ostiole more distinct, the only part yet "carbonized."

C.—Mature pycnidium grown on agar in such a way as to cause inversion of the sporogenous tissue; buffer apparatus penetrated by the pointed ostiole oblique to the surface of the medium.

D.—Fertile tissue completely inverted so that two ostioles are formed.

E.—Mature pycnidium grown on strawberry.

F.—Normal mature pycnidium taken from a leaf of sumac; ring of cell remains lining the central cavity.

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[c] Figure A is from a photograph by Marguerite Ickis.

FURTHER STUDIES ON THE PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE[1]

By B. L. RICHARDS

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In 1917 while at the University of Wisconsin the author began a series of studies on the relation of soil temperature as a factor affecting the pathogenicity of *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn). The results from that part of the work conducted with the potato under control conditions in the greenhouse formed the basis of an early publication.[2] During 1918 and 1919 these temperature studies were extended into the field, where the potato plant and the parasite were grown together under natural soil conditions.[3] It is the purpose of this paper to present the result of these field experiments.

In the greenhouse experiments as reported, several crops of potatoes were grown under controlled conditions in tanks at soil temperatures ranging from 9° to 30° C. The results obtained indicate definitely that while the sterile or "Rhizoctonia" stage of *Corticium vagum* may attack and produce lesions on the potato stems throughout this entire range of temperature it becomes seriously parasitic on this host only at soil temperatures below 24°. Injury to the cortex of the young stem was found especially severe throughout a range of from 12° to 21°, reaching its maximum expression at 18°. Deep cortical lesions, however, frequently occurred at a soil temperature as low as 9°. As explained in the previous report,[2] the most serious type of injury was found to occur at the growing tips of the young sprouts, as these were frequently attacked and killed before they reached the surface of the soil. This particular type of tissue destruction was found especially prominent between 12° and 18°. Above 18° such growing-point destruction decreased rapidly and disappeared completely above 21°. The pathogenic power of the parasite in fact appeared to be so definitely inhibited by soil temperatures above 21° as to render the fungus practically unimportant as a pathogen upon the potato above 24°.

It was further demonstrated in these greenhouse experiments that the young potato shoots grew through uninoculated soil most rapidly at the high temperature of 24° C. For continued growth during the later periods of development of the normal plant, 15° to 18° was found most favorable, while at the high soil temperatures of from 24° to 30° the plants were thrown seriously out of balance and exhibited a number of interesting abnormalities. The rapid growth of the shoots at 21° to 24° was found to be an important factor in the escape of the growing points from injury in the inoculated soil.

[1] Accepted for publication Jan. 16, 1922.

[2] RICHARDS, B. L. PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE. *In* Jour. Agr. Research, v. 21, no. 7, p. 459–482, 5 fig., pl. 88–93. 1921. Literature cited, p. 481–482.

[3] The writer again wishes to express his indebtedness to Prof. L. R. Jones, of the University of Wisconsin, for his helpful suggestions and criticism, and to Dr. W. A. Orton, of the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, for his cooperation in the field work conducted at Plainfield, Wis.

The relative value of these data as an index to the pathogenic action of the fungus under natural conditions of potato culture in Wisconsin was indicated in field studies conducted at Madison and Plainfield.

SOIL INOCULATION WORK AT MADISON, WIS.

During the season of 1919 four successive plantings of Irish Cobbler potatoes were made at approximately 10-day intervals in soil inoculated with the sterile mycelium of *Corticium vagum*. In the process of planting, a pure culture of the fungus grown on a sand-cornmeal medium for four weeks was placed in the soil and two one-half tubers, disinfected before cutting, were planted directly above but in contact with the culture. All sets were then covered to a depth of approximately 6 inches. One plant was grown in uninoculated soil, as a control, for every two plants grown in inoculated soil. In order to obtain an adequate range of soil temperature the first crop was planted at the early date of April 26. The additional successive plantings were made in parallel rows at the various dates of May 7, May 17, and May 30. Observations on the plants in each row were made approximately six weeks after planting. The results of these observations are recorded in Table I. A thermograph record was kept of the soil temperature at a depth of 2 inches [4] for the months of May and June. These temperatures, together with the daily precipitation for the same two months, are recorded in Table II.

TABLE I.—*Effects of Corticium vagum on potato crops planted at various dates through April and May*

Date of planting.	Total hills harvested.	Total stems examined.	Stems slightly injured.		Stems severely injured.		Growing tips destroyed.		Total stems injured.
			Number.	*Per cent.*	*Number.*	*Per cent.*	*Number.*	*Per cent.*	*Per cent.*
Apr. 26	32	182	17	9.3	27	14.8	47	25.8	50.0
May 7	27	202	14	6.9	47	23.2	52	25.5	56.0
17	21	118	19	16.1	14	11.8	8	6.7	34.7
30	25	108	33	30.5	26	24.0	6	5.5	60.0

The result of the experiment shows no specific correlation between the time of planting and the percentage of stems showing injury; on the other hand, the type and intensity of injuries produced by the fungus are evidently very closely related to the date at which plantings were made. As shown in Table I the plants grown from the tubers planted April 26 to May 7 exhibited a high percentage of growing-point destruction as well as a large number of stems which were severely injured. In comparison, the stems grown from the tubers planted on the two later dates of May 17 and May 30 were largely free from growing-point injury and on the whole exhibited lesions considerably less severe than those shown on the plants of the two earlier crops.

A more striking feature of the experiment was noted in the fact that the secondary stems which grew later from the seriously injured primary

[4] Thermograph records of the soil temperature were also kept at a depth of 4 inches for the first two weeks of May. A comparison of the mean daily temperature at the depths of 2 and 4 inches showed an average daily difference for the period of 2.2° C. The daily mean temperatures at 4 inches after May 19 are calculated by subtracting 2.2° from the daily mean at 2 inches. The results are recorded in Table II.

shoots of the first two crops remained practically free from injury (Pl. 1, A, B). The growing tips of these secondary stems showed no indication of injury, while such injury as did occur was very slight and confined entirely to the outer layers of the cortex.

TABLE II.—*Daily soil temperatures and precipitation for Madison, Wis., for the months of May and June, 1919*

Date.	Soil temperature at depth of—				Rainfall.[1]	Date.	Soil temperature at depth of—				Rainfall.[1]
	2 inches.			4 inches.			2 inches.			4 inches.	
	Max.	Min.	Mean.	Mean.			Max.	Min.	Mean.	Mean.	
	°C.	°C.	°C.	°C.	*Inches.*		°C.	°C.	°C.	°C.	*Inches.*
May 1	9.0	6.0	7.5	6.2	0.21	June 1	28.5	19.0	23.7	21.5	0.75
2	.....	.....	.....	8.5	.0	2	27.5	18.5	23.0	20.8	.37
3	9.5	.7	8.2	7.0	.33	3	26.0	19.0	22.5	20.3	Trace.
4	14.8	7.0	10.9	8.7	.21	4	22.0	14.0	18.0	15.8	.39
5	13.8	7.5	10.6	8.5	.0	5	23.0	14.0	18.5	16.3	.01
6	14.8	7.0	10.9	8.0	.63	6	24.1	14.0	19.0	16.8	.04
7	21.2	7.5	14.4	10.9	.0	7	25.5	13.0	19.2	17.0	.01
8	16.2	8.0	11.1	9.4	.0	8	28.0	11.5	20.2	18.0	.0
9	20.0	5.0	12.5	9.2	.0	9	28.2	20.5	24.3	22.1	.18
10	20.9	6.0	13.4	9.8	.0	10	29.8	18.0	23.9	21.7	.18
11	23.6	6.5	16.5	11.1	.0	11	29.5	17.6	23.5	21.3	.74
12	22.9	8.0	15.4	12.6	.0	12	29.0	19.0	24.0	21.8	.0
13	22.8	6.8	14.8	13.9	.0	13	31.0	20.0	25.5	23.3	.02
14	22.5	7.5	15.0	13.5	Trace.	14	30.5	20.5	25.5	23.3	.6
15	17.2	7.8	12.5	10.3	.03	15	21.8	21.0	26.4	24.2	.0
16	19.5	9.0	14.2	12.0	.09	16	30.0	22.0	26.0	23.8	Trace.
17	22.5	7.5	15.0	14.9	.0	17	31.2	22.0	26.6	24.4	.0
18	24.5	7.0	15.7	14.5	.0	18	31.8	21.0	26.4	24.2	.0
19	20.0	10.5	15.2	13.5	.0	19	34.5	21.6	26.0	23.8	.02
20	13.8	7.0	10.4	8.2	Trace.	20	33.5	22.5	28.3	26.1	Trace.
21	.....	.....	.....	.....	.2	21	32.5	21.1	26.8	24.6	Trace.
22	.....	.....	.....	.....	.01	22	29.1	21.0	25.0	22.8	Trace.
23	17.5	10.5	14.0	11.8	.01	23	27.0	21.8	24.4	22.2	Trace.
24	23.2	6.5	14.8	12.6	.0	24	28.8	21.5	25.1	22.9	.05
25	27.8	9.5	18.6	16.4	.0	25	31.8	21.0	26.0	23.8	Trace.
26	27.8	11.4	12.6	10.4	.0	26	27.5	21.8	29.6	27.4	.0
27	30.0	13.0	21.5	19.3	.0	27	23.8	19.5	21.6	19.4	.0
28	30.5	14.2	22.3	20.1	.0	28	30.5	15.2	22.8	20.6	.0
29	32.5	14.0	23.2	21.0	.0	29	32.8	16.0	24.4	22.2	.0
30	32.8	15.0	23.9	21.7	.02	30	3.4	17.5	25.7	25.7	.0
31	32.2	18.0	25.1	22.9	1.72						

[1] The data on the daily rainfall here presented were obtained from the monthly meteorological summary for May and June, 1919, published by the local office of the U. S. Weather Bureau, Milwaukee,, Wis., v. 24, p. 36-37 and 44-45.

For the entire period from April 26 to May 25 an average daily mean temperature of 13.5° C. was maintained at a depth of 2 inches. Fortunately for the purpose of the experiment, between May 25 and 28 the weather turned suddenly warmer and the soil temperature at once arose to an average daily mean of 23.5° (fig. 1). This high daily mean was maintained thereafter until the conclusion of the experiment.

As is evident from the temperature data submitted in Table II, the stems grown from the tubers planted April 28 to May 7 grew through cold soil at temperatures ranging on the average well below 21° C.—the upper temperature limit shown in the greenhouse experiments at which the fungus may attack and destroy the young growing tips. The low temperatures which were maintained up to May 24 so retarded the growth of the young shoots as to afford opportunity for the accumulation of the fungus in the sinus of the young buds, resulting finally in the serious destruction of the growing points noted in the two earlier crops. In view of the very low temperatures maintained during the early part of May it is interesting to recall that growing-point destruction was

found under certain conditions in the greenhouse experiments to reach its maximum expression at temperatures as low as 12°.

In the case of the two later plantings the young potato shoots pushed through the soil at a daily mean temperature approximating very closely the optimum for this early period of growth (24° C.) at which temperature the pathogenicity of the fungus, as previously reported, is definitely inhibited. This increased rate of growth of the host, together with the lessened parasitic action of the fungus at the higher soil temperatures, accounts, in a large measure, for the few growing points injured and for the decreased severity of the cortical lesions noted in the later crops.

The sudden rise in the soil temperature might account in a similar way for the absence of injury to the primordia of the secondary shoots

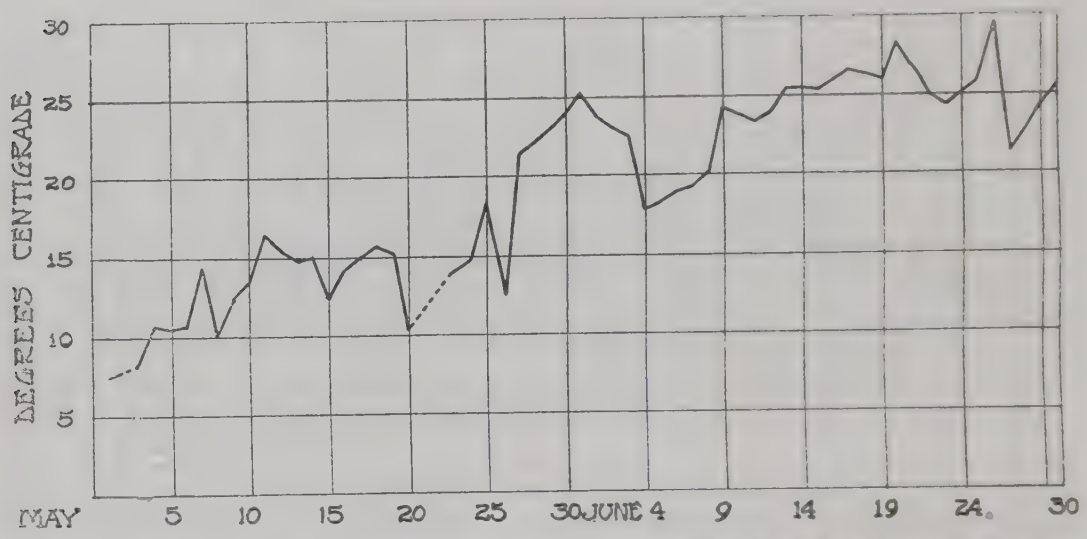

FIG. 1.—Graph showing the range of the mean daily soil temperature at a depth of 2 inches for Madison, Wis., during the months of May and June, 1919. An average mean of 13.5° C. was maintained from April 26 to May 25. Between May 25 and 28 the temperature of the soil increased to a daily mean of 23.5° for the period of May 28 to June 30 (Table II and the discussion, p. 763).

noted in the later two crops (Pl. 1, A, B). The primary stems from these early plantings, as suggested, grew in cold soil which so greatly retarded their rate of growth as to permit girdling and killing of the tender buds. Later with the rise in the temperature, the secondary shoots arising from these injured primary sprouts were permitted to grow so rapidly through the soil as to escape practically uninjured. This is especially significant in view of the fact that the fungus undoubtedly was present in the soil in greater abundance at this later date.

EVIDENCE OF THE IMPORTANCE OF SOIL TEMPERATURE FROM SEED TREATMENT AND PATHOGENICITY TESTS AT PLAINFIELD, WIS.

During 1918 field studies were made at Plainfield, Wis., for the purpose of determining the relation of *Corticium vagum* to seed-potato treatment and to crop development. These studies were repeated at the same place under comparable conditions in 1919.[5] Such uniformity of cultural methods and general field technic were employed for the two seasons as to control as far as possible under field conditions the various factors which might affect the virulence of the fungus. No attempt, however, was made to modify either the soil temperature or the soil moisture content.

[5] The details of these field tests will be published at a later date; only such data as relate definitely to the effects of soil temperature upon the pathogenicity of *Corticium vagum* are included in this paper.

The plots used for the experiments in the two different years were separated by a distance of but a few rods; both were equally level, and the soil was uniform in structure and texture. Seed for the two seasons was obtained from the same stock which had been grown under comparable conditions for a period of seven years on the farm on which the experiments were located. All tubers used for seed were selected with equal care each season for the uniform presence of the sclerotia of *Corticium vagum*. These were cut in halves and planted 5 inches deep at 12-inch intervals in parallel rows 3½ feet apart. The same method of taking observations was employed for the two seasons.

In 1918, 22 rows were planted with 100 hills in each. The seed for 17 of these rows was treated variously with mercuric-chlorid and formaldehyde solution. The remaining 5 rows, placed at regular intervals throughout the plot, were planted with untreated seed for controls. Plantings were made on June 13 and 14.

The first data from the 1918 experiments were taken on July 19, five weeks after planting. At this date a very striking difference appeared between the control rows and the rows grown from treated seed (Pl. 1, C). The plants in the control rows were definitely undersized and irregular; "misses" were frequent, and many of the plants were just breaking soil, while plants in adjacent rows from treated seed were 10 inches or more in height (Pl. 1, C). The examination of alternate plants in the various rows at this time showed severe stem lesions on 80 per cent of the plants from the untreated seed. Numerous primary, secondary, and even tertiary growing tips were destroyed, with total killing of the stems in many instances. The destructive attack on the secondary and tertiary stems is shown in the loss of 16 per cent of the stand and further by the fact that the number of stems per hill from the untreated seed was reduced over 26 per cent as compared with the number from treated seed.

The final results obtained October 12 are equally definite in showing the severity of the disease during the season. Compared with the healthy plants the hills from untreated seed showed an average decrease in the number of tubers produced of 20.7 per cent with a corresponding 34.2 per cent decrease in weight per hill. This average decrease in weight per hill, together with the 16 per cent loss in the stand, resulted in an aggregate loss to the crop from the untreated tubers of approximately 50 per cent.

TABLE III.—*Comparative summary of the results obtained from the Plainfield, Wis., plots during the seasons 1918 and 1919* [1]

Year.	Number of rows.	Number of hills.	Number of control rows.	Number of control hills.	Average hills showing lesions.	Average decrease in stems per hill.	Average decrease in stand.	Average decrease in yield per hill.	Average decrease in tubers per hill.	Average loss to crop.
					Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1918.....	22	2,200	5	500	80.0	26.1	16.0	34.2	20.7	50.0
1919.....	35	3,500	7	700	75.1	Slight Increase.	2.8	12.2	14.3	15.4

[1] The figures in the table are obtained by comparing the plants grown from sclerotia-covered untreated seed with those growing from the same seed equally covered with sclerotia but treated variously with mercuric chlorid.

TABLE IV.—*Daily mean air and soil temperatures and daily rainfall for Hancock,[1] Wis., June, 1918 and 1919*

Date.	Daily mean air temperature for Hancock.[2]		Daily mean soil temperature for Plainfield.		Daily rainfall for Hancock.	
	1918	1919	1918 [3]	1919	1918	1919
	° C.	° C.	° C.	° C.	*Inches.*	*Inches.*
May 26	17.5	20.8	18.5	.....	.....	.....
27	18.0	20.8	19.0	20.6	.....	.....
28	12.2	20.8	13.2	22.0	.....	.....
29	8.8	20.8	9.8	22.5	.....	.....
30	17.2	24.4	18.2	23.1	.....	.....
31	25.0	25.8	26.0	24.5	.....	.....
June 1	21.9	22.7	22.9	23.2	0.30	1.44
2	19.7	21.9	20.7	21.4	.....	1.09
3	17.2	19.1	18.2	19.5	.....	.....
4	16.9	20.8	17.9	19.9	Trace.	.09
5	19.5	18.1	20.5	17.9	.05	.....
6	19.4	15.8	20.4	16.5	Trace.	.09
7	15.0	17.5	16.0	18.2	.....	.43
8	16.1	16.1	17.1	17.3	.04	Trace.
9	18.6	20.3	19.6	20.8	.75	.01
10	17.2	23.1	18.2	21.9	.....	.91
11	23.9	22.8	24.9	21.5	.....	.04
12	19.4	24.7	20.4	23.5	.....	.....
13	17.7	25.3	18.7	24.2	.....	.34
14	19.7	24.7	20.7	25.7	.....	.....
15	17.2	25.3	18.2	26.1	.....	.....
16	26.7	23.9	27.7	27.5	.....	.18
17	23.1	24.4	24.1	24.6	.....	.02
18	16.7	25.5	17.9	26.0	.09	.....
19	19.7	26.1	20.7	26.1	.....	.....
20	18.1	23.3	19.1	23.8	Trace.	.....
21	18.3	.....	19.3	24.3	Trace.	.....
22	14.4	22.2	15.4	23.7	.....	.....
23	16.1	22.8	17.1	22.5	.....	.....
24	22.2	23.6	23.2	22.8	.....	1.00
25	18.5	24.7	19.5	23.1	.....	.05
26	20.3	25.0	21.3	25.0	.....	.....
27	20.3	20.0	21.3	21.5	.....	.....
28	21.1	14.4	22.1	20.7	Trace.	.....
29	19.7	18.3	20.7	22.0	Trace.	.....
30	18.6	22.8	18.6	.....	1.26	.....
Mean average	19.1	22.0	20.1	23.0	.....	.....
Total rainfall for June	.....	.....	.....	.....	2.49	5.69

[1] The soil temperatures were determined and calculated for a depth of 4 inches.
The nearest weather recording station to Plainfield was at Hancock, Wis., 6 miles southwest. The soil and weather conditions at the two places are in general very similar. The average daily mean soil temperature at a depth of 4 inches at Plainfield during 1919 was found to be just 1° C. higher than the average daily mean air temperature recorded for the same day at Hancock. Assuming the same relation to hold true for 1918, a fair index is obtained for calculating both the soil and the air temperature at Plainfield for the season of 1918.

[2] The daily air temperatures for Hancock for May and June, 1918 and 1919, were calculated directly from the Wisconsin section of the Climatological Data, Weather Bureau, United States Department of Agriculture.

[3] Calculated.

In 1919, 35 parallel rows each containing 100 hills were planted as in 1918. Seven of these rows were planted with untreated seed as controls. Plantings were made May 25 and the first observations on July 3, five weeks later. At this later date the ravages of the disease were not nearly so apparent as in 1918. While the control rows could be detected

with little difficulty by the irregular size of the plants, yet "misses" were infrequent and many of the control plants were equal in size to those from treated seed. Fewer growing points were destroyed than was noted in 1918, while few, if any, secondary stems were seriously attacked. The lesions on the whole were much less severe than those of the previous year, as is shown by the fact that no reduction occurred either in the stand or in the number of stems per hill in the diseased as compared with the healthy hills. The relative severity of the disease is further shown by the smaller decrease in the yield from the diseased hills, as indicated both in the weight and number of tubers per hill. In every respect the action of the fungus was definitely less severe during 1919 than during 1918. The comparative results are shown in Table III.

As stated, the most important uncontrolled factors operating during the two seasons of 1918 and 1919 were soil temperature and soil moisture. The precipitation was much lighter during the first four weeks after planting in 1918 than during the same period of 1919, as is shown in Table IV. As shown in Table V, during June, 1918, 2.49 inches fell, as compared with 5.69 inches during June, 1919. For the second, third, and fourth weeks of growth in 1918 and 1919 the rainfall was 2.24 and 4.23 inches, respectively. It should be noted, however, that during the month of May, 1918, 9.78 inches fell, while 4.08 inches of rain fell in May, 1919. This early rainfall no doubt influences greatly the amount of moisture in the soil during this early part of the crop development.

TABLE V.—*Monthly mean of daily temperature and total monthly rainfall for Hancock, Wis., during the potato-growing season of 1918 and 1919*

Month.	Average daily mean air temperature.		Total rainfall.	
	1918	1919	1918	1919
May	16.0	13.1	9.78	4.08
June	19.1	22.0	2.49	5.69
July	21.25	23.2	1.92	2.13
August	22.7	19.0	3.12	2.31
September	12.75	.....	1.31	.....

By far the greatest damage to the crop occurred during the season of least rainfall. While little positive evidence is available as to the exact effect of moisture on the pathogenic action of *Corticium vagum* on the potato, it has been generally assumed to be more virulent in relatively damp soil. It is hardly probable that the decreased rainfall during 1918 functioned as an important factor in the greater damage done by the fungus during that year. It is suggested in this connection that the low moisture content of the soil during 1918 might have acted as a retarding factor in the growth of the young shoot through the soils, thus contributing to the severity of the disease. No indication of such retardation, however, was noted. In fact, the crop on the plots in general exhibited greater vitality during the early season of 1918 than during the same period of 1919.

In view of the known facts the relation of soil temperature to the variation in the severity of the disease during 1918 and 1919 appears more clear.

Fortunately, again, for the purpose of the experiments the temperature for the two years differed widely. June, 1918, was cool, averaging 0.11° C. below normal for the locality, while June, 1919, was the warmest that had been experienced in Hancock and surrounding districts for a long period of years, with an average of 2.6° above normal. This high average, it is evident from the figures submitted in Table IV, was due to uniformly high temperatures rather than to any period of excessively high temperatures. Mr. H. B. Hersey, Director of the Wisconsin Section of the U. S. Weather Bureau, states that—

> June was unusually warm. The average temperature for the State, 69.4° F., has been exceeded only once in June since records began in 1891.

The actual daily mean air temperature for Hancock for the month of June was 19.1° C. in 1918 and 22° for the same month in 1919. The average mean daily soil temperature of 23° was determined for Plainfield during June in 1919 with a calculated soil temperature of 20.1° for 1918 (Table IV), or a difference of 2.9°. For the second, third, and fourth weeks after planting the average daily mean temperature calculated for 1918 was 19.4° with an actual daily mean of 22.8° for 1919, a difference of 2.4°.

These temperature relations are especially significant when considered in view of the critical temperature for growing-point injury determined in the more carefully controlled greenhouse experiments. As previously indicated, no damage was found to occur to the growing points of the young potato shoots above 21° C., while below this to as low as 12° serious destruction of the young bud resulted. In 1918 the average mean soil temperature during the critical period of growth the shoots through the soil remained below this critical temperature, while in 1919 the average daily mean was well above 21°. Growing-point destruction was almost wholly responsible for the reduced stand and the decreased number of stems per hill in the control rows in the 1918 crop. It is also significant that the average daily mean of 20.1° maintained during June of 1918 approaches closely the soil temperature (18°) found optimum for the destruction of cortical tissue of the young stems, while during June, 1919, which included the entire time which the young shoots were growing through the soil, the average daily mean soil temperature approximated closely that temperature (24°) found in the greenhouse experiments to be optimum for the early growth of the young potato shoots and at which temperature the fungus, due to some inhibitive factor, ceases to be seriously parasitic on potato stems. In view of the facts, it appears evident that the greater damage to the 1918 crop, amounting to three times that of 1919, was occasioned primarily by low soil temperature during that season. The high soil temperature, on the other hand, appears equally responsible for the inhibited action of the pathogen during 1919.

DISCUSSION

Results obtained from both the field and greenhouse experiments emphasize clearly the controlling influence of soil temperature upon the pathogenic power of *Corticium vagum*. While the fungus, as was shown in the earlier studies, may produce lesions on the underground parts of the potato over a relatively wide range of soil temperature, it has become evident that variations in the average mean soil temperature of 2° or 3° above or below 21° C. and maintained for the first few weeks after planting may determine accurately the degree to which this fungus may

damage the potato crop. However, from the standpoint of practical methods of control, except for the possible advantage gained by late planting, we may expect but little from temperature relation of the fungus. This is the more evident considering the fact, as pointed out in the earlier publications, that the fungus operates most vigorously as a parasite under such temperature as best favors maximum potato production.

The generally accepted idea borne out by these field experiments that the early crop of potatoes is more severely damaged by *Corticium vagum* appears to be largely a question of the cooler soil temperatures maintained during the early growth of the plants. What part may be played by the higher soil moisture content generally occurring during these earlier dates remains undetermined. Whether such increased soil moisture, if important, contributes directly to the pathogenic activity of the fungus or only indirectly by its influence on the temperature of the soil is not clear. From the greenhouse experiments in which the moisture factor was carefully controlled, it would appear evident that such variations as frequently occur in the temperature values between early and late planting might adequately account for such differences as are noted in the pathogenicity of the fungus in question. Again, the greater susceptibility to the attack of *C. vagum* frequently attributed to our standard early varieties enters in as a possible factor in the greater damage to the early crops. Here again it is not improbable that the suggested susceptibility is induced by the particular environmental conditions to which these early varieties are exposed. The possible increased virulence of the fungus produced by a cooler soil evidently functions here as an important factor.

Such determination of the various temperature relations of *Corticium vagum* made during these studies may add much by way of explanation of many of the conflicting opinions held regarding the pathogenicity of the fungus as a potato parasite. Seasonal variation in the severity of the stem canker is easily explained in view of the facts presented. Special significance is also given to the observation that the stem canker caused by *C. vagum* reaches its maximum severity in the northern potato sections of Canada and the States of Maine, Wisconsin, and Michigan, as also in the higher altitudes of the Western States. Clearly, however, the fungus may become especially virulent on the very early crops even in the warmer areas.

SUMMARY

(1) Special significance is given to the soil temperature relations of *Corticium vagum* established in the earlier greenhouse experiments by the field studies conducted during 1918 and 1919. These studies further emphasize the importance of the soil temperature as a controlling factor in the pathogenicity of the sterile stage of *C. vagum* on the potato.

(2) *Corticium vagum* caused greater growing-point destruction and a more severe type of cortical injury on the early potato crop planted April 26 and May 7 than on the later plantings of May 17 and 30. This difference was due to a sudden rise in the soil temperature at such dates as to permit early growth of the later crops to take place in a very warm soil.

(3) Growing tips of the young primary shoots grown from potato tubers planted at the early dates of April 26 and May 7 were destroyed in cold soil by *Corticium vagum*, while secondary primordia developed from such injured shoots later grew through warm soil uninjured.

(4) Greater damage to potato plants occurred in the field in sclerotia-inoculated soil during the cool season of 1918 with a daily mean soil temperature for June of 20.1° C. than during 1919 with an average daily mean of 23° for the same growth period. This loss in 1918 was expressed in a greater decreased stand, a greater number and a more severe type of stem lesions, fewer stems per hill, decreased number of tubers per hill, and a total loss in yield of 50 per cent as opposed to a total loss of 15.4 per cent in 1919.

(5) The results in general indicate that the occurrence of an average daily mean temperature of 2° above or below the critical temperature (21°) for the pathogenicity of the *Corticium vagum* on the growing points during the first six weeks of potato growth may accurately govern the damage done to the crop by this fungus in any one season.

(6) Such temperature data as are here presented will go far in helping to explain many of the conflicting views held regarding the pathogenicity of *Corticium vagum* on the potato and other hosts.

PLATE I

A.—Irish Cobbler potatoes grown under field conditions in soil inoculated with a pure culture of *Corticium vagum*. Plant grown from tuber planted April 26, showing the destruction of the primary growing point and uninjured secondary stem. Twenty-five per cent of the growing points of the primary shoots of the crop planted on this date were destroyed (Table I).

B.—Same as A except that plant was grown from tuber planted May 7. A total of 25.5 per cent of the primary buds of this crop were destroyed.

The photographs illustrate how the growing tips of the primary shoots growing at the lower temperatures were attacked and killed by the fungus. Later secondary shoots arose from the primary stems which with the advent of higher soil temperatures grew so rapidly through the soil as to escape uninjured. The decreased pathogenic power of the fungus at the higher temperature no doubt aided in this escape.

C.—Potato plot at Plainfield, Wis., showing the disastrous effects of *Corticium vagum* on the potato crop during the cool season, 1918. The third row from the right was planted with sclerotia-covered, untreated tubers. The plants in the rows on either side were grown from tubers equally "scurfed" but treated with mercuric chlorid.

A
B
C

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INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL[1]

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INTRODUCTION

It is the purpose of this paper to present the results obtained during the past three years in the investigations on the rosette disease of winter wheat. This disease was reported from Madison County, Ill., in the spring of 1919 by Lyman[3] and later it was found in other counties in Illinois and in several counties in Indiana. Shortly after its discovery it was described by Humphrey and Johnson (*9*).[4] At that time the disease was considered apparently identical with the take-all disease occurring in Australasia and the footrot disease occurring in Europe. However, investigations herein reported show that the rosette disease of wheat differs from take-all in plant symptoms and also in the varietal and host ranges. Furthermore, none of the fungi which are commonly found associated with take-all abroad and in parts of this country have been found associated with the rosette disease. It is therefore clearly evident that the latter disease occurring in Illinois and Indiana is distinctly different from the take-all disease.

The take-all, footrot, and similar diseases which occur abroad also occur in several States in this country.[5] While the causal agent may or may not be the same in all cases, the plant symptoms manifested in

[1] Accepted for publication Oct. 28, 1921. In this paper the designation rosette disease will be applied to the wheat disease occurring in Illinois and Indiana, previously referred to as take-all and so-called take-all. The name take-all will be used in referring to the disease caused by *Ophiobolus graminis* Sacc. The diseases producing symptoms more or less closely similar to take-all will be grouped under the name footrot. The investigations upon which this paper is based were carried on in cooperation with the Wisconsin, Illinois, and Purdue University Agricultural Experiment Stations, the Missouri Botanical Garden, and the Board of Education, Granite City, Ill.

[2] Especial credit is due Prof. G. H. Dungan, of the Illinois Agricultural Experiment Station, for bringing together certain fertilizers and seed samples used and for assisting in sowing some of the plots and in other ways, and Dr. C. E. Leighty, Agronomist, Office of Cereal Investigations, United States Department of Agriculture, for identifying wheat varieties and for furnishing all seed marked "C. I." in the tables. The writer is indebted to Prof. L. R. Jones and Dr. A. G. Johnson for helpful suggestions throughout the work herein reported.

[3] LYMAN, G. R. "TAKE-ALL" OF WHEAT IN THIS COUNTRY. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Survey Circ. 1 p. May 1, 1919. Mimeographed.

[4] Reference is made by number (italic) to "Literature cited," pp. 799–800.

[5] A wheat disease of the take-all type was first reported in America from Oregon in 1902 by Cordley (*3*). None of the organisms which are associated with this type of trouble abroad were reported by him, however, and this type of disease was not reported again from Oregon until 1921 (*36*).

In 1919 the same type of wheat malady was reported from Roanoke County, Va. (*20*). Diseased plants sent to the Office of Cereal Investigations, United States Department of Agriculture, by Dr. F. D. Fromme on June 16, 1919, showed typical symptoms of take-all and footrot as they are described in the foreign literature. Examinations made by the office staff revealed the presence of mature perithecia of *Ophiobolus graminis* Sacc., the organism which is now known to cause take-all. Later this disease and organism were found in Washington (*6*), New York (*14*), Arkansas (*36*), Oregon (*36*), and Knox County, Ind. (*36*).

This type of malady associated with *Leptosphaeria herpotrichoides* de Not has been found in Washington by the writer and with *Wojnowicia graminis* (McAlpine) Sacc. and D. Sacc. (*21*) in Kansas, Arkansas, and Oregon.

different localities have been observed to be very similar and in general they answer the descriptions for take-all and footrot as given in the foreign papers by Prillieux and Delacroix (*26*), Mangin (*22*), McAlpine (*19*), Foëx (*4*), and Waters (*37*).

The rosette disease of wheat was not reported until 1919; however, many farmers in the infested areas are certain that they have observed the trouble in wheat for many years. While such reports, of course, are not entirely conclusive, they indicate that the trouble may have been in existence in certain of the infested areas for a considerable length of time. The fact that the disease has recurred each year since it was reported in 1919 adds weight to this possibility. Furthermore, all attempts to establish its origin through channels of introduction have failed, indicating still further that the introduction of the disease has not occurred recently.

As early as 1802 a destructive wheat disease was reported by Hollingsworth (*7*, *8*), and Mease (*25*) [6] from the district near Elkton, Md. In some ways the descriptions of this malady fit the symptoms for the rosette disease, while in other respects they seem to fit those for the true take-all. The descriptions of this trouble are not adequate, making it difficult or impossible to tell exactly whether or not it was the same as the rosette disease. Further light may be thrown on the matter of origin after the cause of the rosette disease has been determined.

COMMON NAME OF THE DISEASE

Shortly after the discovery of the disease in Illinois, the name take-all was applied to it by Humphrey and Johnson (*9*). Later, Stevens (*33*) designated the disease by the name footrot; and Johnson and Haskell (*12*) and McKinney (*20*) [7] have designated the trouble by the tentative name "so-called take-all." Since all of the evidence indicates that the disease is not the same as the Australian take-all, obviously the name take-all is not an appropriate one for it. With reference to the name footrot the American and the foreign literature shows that as a common name footrot is closely associated with the name take-all. This conception is confirmed further by plant pathologists from Australasia and Europe with whom the writer has conferred.[8] McAlpine (*19*), Massee (*23*), and Dana (*2*) consider these names to be practically synonymous. In view of this relationship, the writer prefers not to accept the name footrot for the Illinois disease. It, therefore, seems advisable to designate the disease by the more descriptive name "the rosette disease of wheat."

[6] These rather obscure references were brought to the attention of the writer through the kindness of Dr. G. H. Coons of the Michigan Agricultural Experiment Station.

[7] McKINNEY, H. H. RESISTANCE IN WHEAT TO THE SO-CALLED TAKE-ALL DISEASE. *In* U. S. Dept. Agr. Bureau Plant Indus. Off. Cer. Inves., Cereal Courier, v. 12, no. 17, p. 229–231. 1920. Mimeographed.

[8] Dr. A. D. Cotton, Mycologist to the Board of Agriculture, Kew, England; Dr. E. T. Foëx, Director of Station of Vegetable Pathology, Paris; Dr. A. H. Cockayne, Government Biologist, New Zealand Department of Agriculture, Wellington, New Zealand; Dr. W. L. Waterhouse, University of Sydney, Sydney, New South Wales; W. R. Birks, Chatswood, New South Wales.

DISTRIBUTION

So far as definitely known, the rosette disease of wheat occurs only in the States of Illinois and Indiana. Table I gives the counties in which the disease has been located and the number of fields and areas infested in each.

TABLE I.—*Distribution and severity of the rosette disease in the United States in 1919*

State.	County.	Number of fields infested.	Acreage in infested fields.	Extent of disease in infested fields.
Illinois	Madison	25	629	Trace to 85 per cent.
	Mason	48	1,310.5	Trace to 70 per cent.
	Sangamon	2	380	Trace to 5 per cent.
	Logan	1	.....	
Indiana	Laporte	5	150	Trace.
	Porter	6	120	Trace to 63 per cent.
	Tippecanoe	1	5	Trace.

ECONOMIC IMPORTANCE

While the occurrence of this trouble is restricted to relatively small areas, its importance must not be overlooked. Under ideal conditions for the disease it has caused as high as 40 per cent actual loss of grain in a 50-acre field. It is not uncommon to find many spots or large areas in a field where practically all the wheat plants are killed. Sometimes, however, the diseased plants in such spots recover to a considerable extent and produce some grain. However, such plants usually are so delayed in ripening that they are green when the normal crop is harvested. The grain from such green plants shrivels badly, and much of it is lost in thrashing. When much of a field is affected by the disease and there has been considerable recovery, the greatest quantity of grain is saved by delaying harvest until the diseased areas are ripe. It frequently is more economical to take a small loss, due to the shattering of overripe healthy plants, than to harvest the recovered plants while green. In some cases, where diseased areas in a field are more or less segregated, it is possible to harvest the healthy and diseased areas separately, thus reducing losses to the minimum.

HOST PLANTS

Investigations carried on thus far have not shown any other crop than wheat to be positively affected by the rosette disease. Results of attempts to infect various plants will be found in Table II. There is some suggestion that rye may be infected, but this is not certain. While further investigations are being conducted to throw additional light on the host range of the disease, it is considered unnecessary to include plants outside the grass family until more is known about the cause of the disease.

TABLE II.—*Percentage of rosette disease in various crops planted on infested land, Granite City, Ill., in 1919*

Crops.	Percentage of roestte disease.	Crops.	Percentage of rosette disease.
Winter wheats listed in Tables VII and VIII (1920–21)	0 to 98	Buckwheat	0
Marquis spring wheat	?	Flax	0
Durum spring wheat	?	German millet	0
Illinois No. 1 spring wheat	?	Hungarian millet	0
Red Fife spring wheat	?	Barnyard millet (Billion Dollar grass)	0
Spring emmer	0	Timothy	0
Winter emmer	0	Awnless rye-grass	0
Spelt	0	Italian rye-grass	0
Hanna barley	?	English rye-grass	0
Hannchen barley	?	Bermuda grass	0
Svanhals barley	?	Crested dog's-tail	0
Wisconsin Pedigree barley	?	Meadow foxtail	0
Rosen rye	0	Meadow fescue	0
Common rye	0	Red fescue	0
Spring rye	0	Red top	0
Texas Red oats	0	Tall meadow oat-grass	0
Big Four oats	0	Kentucky bluegrass	0
Iowa 105 oats	0	Spring vetch	0
Sixty Day oats	0	Alfalfa	0
Iowa 103 oats	0	Sweet clover	0
Corn (maize)	0	White clover	0
Kafir	0	Cowpea	0
Feterita	0	Navy bean	0
Amber sorgo	0	Rape	0
Milo	0	Sunflower	0
Sudan grass	0	Giant beggarweed	0

Certain varieties of spring wheat and of barley develop symptoms resembling the rosette disease of winter wheat, but there are certain differences which make it questionable whether these crops are affected by the disease.

DESCRIPTION OF THE DISEASE

During the first year's investigations it was not possible to make a complete study of the symptomatology of the disease, owing to the fact that the work was not started until May, fully seven weeks after the disease made its appearance in the spring. At that time field spotting was rather evident, and diseased plants showed a characteristic stunted development and the production of an excessively large number of secondary tillers, giving the plants a rosette appearance. These were the field and plant symptoms which attracted attention to the disease and which made it conspicuously different from other wheat diseases heretofore definitely known in this country. A rotting of the underground portions of tillers had started, but this condition was suspected to be a secondary effect rather than a primary cause, as observations, made earlier in the season (April 21) by A. G. Johnson, had not revealed a distinct rotting of the tissues in all cases, even though conspicuous signs of the disease, dwarfing and excessive tillering, were noted by him.

In 1920 the field studies were begun in the latter part of March, just at the time wheat plants were beginning to show signs of spring development. These studies were conducted in experimental plots located near

Granite City, Ill. In 1920 the first signs of the disease became manifest on March 24, and by April 1 there was no question as to its presence.

During 1921 observations were made on the experimental plots located near Granite City, Ill., and also on plots maintained by the Indiana Agricultural Experiment Station near Valparaiso, Ind. In 1921 the disease made its appearance at Granite City during the latter part of February. This early development doubtless was due to the fact that the winter had been unusually mild. The disease has not yet been detected with certainty in the fall.

FIELD SYMPTOMS

Shortly after spring growth begins diseased areas present a very striking contrast with healthy parts of the field. Such areas show up as rather definite patches which vary in size and shape (Pl. 2). In some cases one diseased plant (Pl. 3, A) may be found among plants showing no signs of the disease. Frequently patches are found which are made up of only a dozen or so diseased plants, but usually the patches are considerably larger than this, and most commonly range from 3 feet to 20 feet or more in diameter. These patches sometimes are circular, but more commonly are irregular in outline. Where the disease is most destructive, more than 95 per cent of the area of the field may be infested. The marginal limits of diseased areas tend to be rather sharply defined in contrast with the rather undefined patching usually found in connection with poor soil conditions. The spotting caused by the disease is independent of topographical variations, and frequently patches are found to cover both poorly drained dead furrows and adjacent well-drained land. In the diseased patches, plants which partly recover tend to ripen later than healthy ones, causing a striking green spotting in the fields at ripening time. During wet seasons weeds become abundant in the disease-infested spots.

PLANT SYMPTOMS

The first positive indication of the disease, as the latter is now interpreted, consists in a retarding of the early spring development of the fall tillers. At this time no external lesions are consistently associated with the living tissues of the diseased plants (Pl. 1, B). Occasionally Helminthosporium lesions have been found on the subcrown internode.[9] As pointed out later, such lesions sometimes are discernible in the early spring on the dead sheaths of autumn leaves.

In the course of about a week after the first appearance of the rosette disease, affected plants tend to develop a dark, rather bluish green color, and numerous secondary tiller buds and tillers develop. The development of these secondary buds and tillers takes place around the crown of the plant, in a manner somewhat analogous to the twig proliferations occurring in peach rosette as described by Smith (*31*). The excessive number of tillers developed by diseased plants give them a characteristic rosette appearance. Table III gives results of tiller counts made on the healthy plants and plants affected by the disease.

[9] The term subcrown internode as used in this paper refers to the structure in the wheat seedling between the crown and the base of the coleoptile. Under ordinary conditions this internodal structure usually elongates to a greater or less extent.

TABLE III.—*Comparison of tillering in healthy wheat plants and those affected by the rosette disease of wheat*

Variety.	Locality.	Date of observations.	Total number of plants counted.	Average number tillers per plant.	
				Diseased.	Healthy.
Harvest Queen (Salzer's Prize-taker) [1].	Madison Co., Ill..	Apr. 7, 1920	48	10.0	4.5
Harvest Queen (white-chaffed Red Cross).[1]	Porter Co., Ind...	May 7, 1921	54	9.2	3.3

[1] Local names: The white-chaffed Red Cross and the so-called Salzer's Prize-taker are considered to be the same variety grown locally under the different names. The preferred varietal name for these is Harvest Queen. The name "Salzer's Prize-taker" as used in this paper refers to the variety with white chaff and red kernels grown under the this name in southern Illinois. The original or true Salzer's Prize-taker is a variety with red chaff and white kernels. (See p. 794.)

At the time these spring tiller proliferations become pronounced the basal tissues of the fall tillers begin to take on a dull white to straw color, and in the course of another week or 10 days this tissue may start to turn brown and the whole base of the plant may become rotted (Pl. 1, C). This last condition seems to vary considerably in different localities under different conditions. In the vicinity of Granite City, Ill., this rotting is apparently much more prevalent than it is near Valparaiso, Ind.

Microscopic examinations [10] of the innermost tissues at the bases of stunted tillers of plants showing the early symptoms of the rosette disease have revealed the presence of yellowish brown necrotic areas located within the parenchymatous region. These necrotic areas seem to be similar to those found in the stems of corn plants and in sugar-cane plants affected by mosaic, as reported by Kunkel (*15*) and Matz (*24*), respectively. Examinations of the host cells in and adjacent to the necrotic areas frequently show intracellular bodies very similar to those found in tobacco, sugar cane, and corn plants affected by mosaic, as described by Iwanowski (*11*), Matz (*24*), and Kunkel (*15*). These bodies are also very similar to those described and figured by Lyon (*17*) in connection with the Fiji disease of sugar cane.

Root systems of plants in the early stages of the disease do not show external signs of infection or other injury consistently. In some cases lesions have been found on roots, but fully as many have been found on the roots of plants not showing the symptoms of the rosette disease. As the disease progresses the root systems of affected plants show a retarded development and become infected to some extent by various organisms.

In dry situations diseased plants do not tend to send up secondary spring tillers, and such plants usually die early, forming a drooping tuft of brown, dead leaves and tillers (Pl. 3, B). During periods of heavy rain in the spring, diseased plants may be washed and beaten out of the soil, leaving the ground bare between healthy plants. In situations of favorable moisture and high fertility diseased plants often recover. While the fall tillers of such plants usually are killed, the secondary tillers may develop into short culms (Pl. 4, B) and produce heads. Such heads usually are small and imperfectly filled (Pl. 4, A).

[10] Much of the microscopic work done in connection with this phase was carried on by Dr. Sophia Eckerson and Dr. R. W. Webb. A full account of this work will be published in a future paper.

Stevens (*33, p. 259*) recognized the development of extra tillers or "shoots" as a characteristic of the rosette disease (called footrot by him), but apparently he did not regard this as a primary characteristic. He considered the browning and rotting of the basal portions of the plants as the most "constant" character of the disease.

It is quite true that when the rosette disease is not observed until a considerable length of time after its first appearance in the spring, the darkening and rotting of the basal tissue might be considered, under certain conditions, as constant characters, but even these characters do not seem to hold in all localities where the disease occurs.

When the rosette disease, as it occurs in different localities, is taken into consideration, the most constant or characteristic symptoms which have been found in three years of close observation, consist of (1) the arrested spring developments, (2) the excessive tillering, producing a rosette appearance, and (3) the dark blue-green color of the foliage in combination with the characters mentioned above.

On the basis of our present knowledge, the presence of Helminthosporium lesions on, or a rotting of, the basal parts of wheat plants can not by themselves be considered diagnostic characteristics of the rosette disease. This Helminthosporium is found to cause considerable injury on many wheat varieties growing in the regions where the rosette disease occurs and which are not known to show the characteristic symptoms of rosette. Furthermore, this organism has been found attacking wheat in sections of the United States where the rosette disease is unknown.

It is not the purpose of this paper to deal with the take-all and footrot type of diseases occurring in the United States, but it does seem advisable to give the principal characteristics of these troubles as found in this country, in order that they may be distinguished from the rosette disease.

The field spotting caused by take-all and footrot is practically the same as that caused by the rosette disease. Field spots, however, tend to show up earlier in the spring in the rosette disease than in take-all and footrot. The first indication of the latter seems to be a yellowing of the affected plants, a condition not found in the rosette disease. Such yellow plants rapidly bleach out or develop a bronze color and die, after which they tend to remain erect in a rather stiff, upright position, or they may break over at the base due to a dry crownrot. When death is caused by the rosette disease, the plants turn brown without passing through the yellowing stage, and such plants droop to the ground and form a flat tuft of brown, dead leaves.

A black plate or scalelike mat of mycelium is commonly found at the base of the tillers of plants affected by the take-all and footrot type of maladies, but this condition has never been found associated with the rosette disease. Premature ripening or the development of "white heads" occurs in the former, but this does not occur in the rosette disease. Rather the opposite condition takes place—that is, delayed ripening of the partially recovered diseased plants.

In general it may be said that the rosette disease of wheat may be diagnosed with certainty in the spring before healthy plants reach the boot stage. After this period the disease may, under certain conditions, be indistinguishable from certain insect and other troubles. The comparative symptomatology of some of the insect maladies and the rosette disease will be taken up in a later paper prepared jointly between the

Bureaus of Plant Industry and Entomology, United States Department of Agriculture.

In certain particulars the early stages of the nematode disease of wheat resemble the early stages of the rosette disease. In each disease there may be retarded development of the plants in the spring and the leaves may develop a dark green coloration and become very broad and thick, but in nematode-infested plants there is, in addition, usually a marked crinkling of the leaves and stems, a condition not associated with the rosette disease. Also, the leaf and stem tissues of nematode-infested plants are very vegetative and succulent in nature, and they show a characteristic sparkling surface. None of these characters are associated with the rosette disease.

In reviewing the literature [11] it was found that certain diseases of sugar cane show marked resemblances in certain particulars to wheat rosette. The Fiji disease as described by Lyon (*17*) is especially similar, and likewise the Sereh disease described by the same writer has certain characteristics in common with the rosette disease.

In certain particulars the mosaic disease of cane and that of corn resemble the rosette disease even in the matter of leaf mottling. The latter character has always been observed by the writer in connection with wheat rosette, but since it also occurs in varieties not susceptible to rosette it is not known whether the condition is directly connected with the rosette disease. Evidence in hand suggests that there may be a connection.

CAUSE OF THE DISEASE

It should be noted that the development of typical plant symptoms of the rosette disease seems to be connected in some way with dormancy or low temperatures which occur during dormancy. When plants are not subjected to these conditions the characteristic plant symptoms do not ordinarily develop. Shortening the dormant period has given fair results, but until methods are perfected it will not be possible to make much progress by working under greenhouse conditions or by working in the open out of the regular season for the development of winter wheat.

While a number of theories have been advanced concerning the cause of the rosette disease, the exact cause is still undetermined. The disease has been variously attributed to winter injury and to abnormal soil conditions, such as depleted fertility, presence of toxic or injurious substances, unfavorable soil type, and improper drainage. Animal and plant parasites and viruses have also been considered the cause. Most of these possible causal agents have been tested either directly or indirectly, and certain conclusions have been reached. These agents are discussed under the general divisions (1) nonparasitic factors, (a) winter injury, (b) soil conditions other than parasitic; (2) parasitic factors, (a) animal, (b) plant; (3) viruses.

NONPARASITIC FACTORS

WINTER INJURY

During the season that the rosette disease was first reported (1919) it was generally considered, among many farmers, that the trouble was caused by the abnormally mild winter of 1918-19. While there is

[11] The writer is indebted to Dr. C. W. Edgerton for the use of his library in connection with these studies.

evidence that winter conditions do influence the development of the disease, there is no evidence that winter conditions are the prime cause. While the disease was first reported after an unusually mild winter, it has recurred each spring since that time. In this 3-year period, two mild winters and one severe winter have occurred, as shown in figure 1.

This figure shows the average monthly mean temperature curve for the 10 years previous to 1918 and the monthly mean temperature curves for the past 3 winter seasons in the vicinity of Granite City, Ill. These curves are based upon the daily temperature records published by the United States Weather Bureau at St. Louis, Mo.

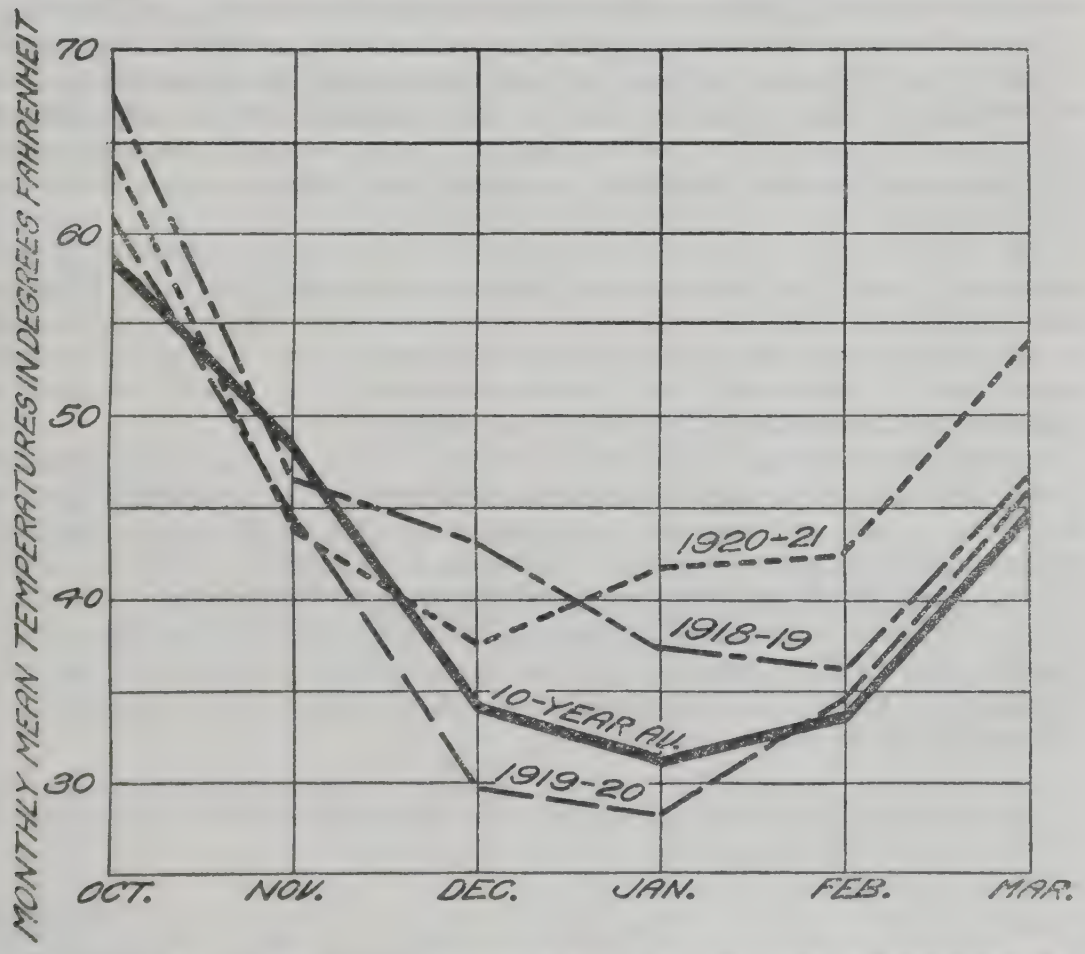

FIG. 1.—Graph showing monthly mean temperatures in degrees Fahrenheit for the six winter months in the years 1918–19, 1919–20, and 1920–21, compared with the 10-year average temperature in the same six months. The winters of 1918–19 and 1920–21 were unusually mild.

During the winters of 1918–1919 and 1920–1921 there was very little snow cover, and, as shown by the curves (fig. 1), these seasons were above normal in temperature. During the winter of 1919–1920, however, the opposite was true.

These data, together with the results from soil sterilization experiments discussed under Parasitic Factors on page 783, show that weather conditions in themselves are not the direct cause of the rosette disease.

SOIL CONDITIONS OTHER THAN PARASITIC

The rosette disease has been found to occur on all types of soil from a poor sand to a reasonably fertile gumbo, and under all conditions of soil drainage.

From the beginning of these investigations these factors were not considered very promising possibilities as primary causes of the disease;

first, because of the wide soil variations under which the disease occurred, and second, because characteristic symptoms of the diseased wheat plants are unlike the symptoms manifested by wheat plants suffering from a lack of fertility or from poor drainage. Plants suffering from the latter conditions usually make a poor start in the fall, and tillering is greatly reduced compared with that of plants growing in properly drained, fertile soil. Both of these symptoms differ from those found with the rosette disease.

While these observations practically eliminate soil type and drainage factors from the possible direct causes of the disease under discussion, they serve only as indirect evidence concerning the matter of soil fertility.

From the standpoint of nutrition and of soil toxins which may result from decomposition of organic or inorganic matter, or from plant root excretions, it was considered that if these factors were by any chance the primary cause of the rosette disease, the fact could be demonstrated by conducting simple fertilizer, cropping, and tillage experiments on infested land.

In light of the modern conception held by many workers in soil technology and allied branches, as Truog and Sykora (*35*), Lyon, Fippin, and Buckman (*18 p. 136–138*), Schreiner and Skinner (*30*), and Livingston (*16*), it was believed that some one of such a series of experiments would correct any soil disorders which might cause the disease and thus in turn materially control the disease.

In the fall of 1919, eight plots (30 by 44 feet) of uniformly infested land were used for the soil-treatment experiments.[12] Applications of fertilizers, lime, and manure were made on five of the plots, and the remaining three plots were left untreated for controls. All treatments were applied in the fall, either just before or just after seeding. Harvest Queen (Salzer's Prize-taker) wheat seed from a field free from the rosette disease was drilled across all of the treatments. Table IV gives the treatments, the rates of application of the materials, and the extent of disease which developed in the plots.

TABLE IV.—*Data showing little influence of fertilizers, manure, and lime upon the control of rosette disease in Harvest Queen (Salzer's Prize-taker) wheat sown on uniformly infested land at Granite City, Ill.*

Series.	Plot No.	Treatment.	Rate per acre.	Percentage of plants diseased.
1919–20	1	None		90
	2	Acid phosphate	222 pounds	92
	3	Rock phosphate	1 ton	97
		Stable manure	6 tons	
	4	Finely-ground lime stone	2 tons	93
		Rock phosphate	1 ton	
		Stable manure	6 tons	
	5	None		94
	6	Finely-ground lime stone	2 tons	96
	7	Stable manure	6 tons	93
	8	None		95
1920–21	1	None		85
	2	Potassium sulphate	500 pounds	85
	3	Sodium nitrate	440 pounds	87
	4	Acid phosphate	250 pounds	84
	5	Finely-ground lime stone	2.5 tons	88

[12] All the field plot experiments referred to in this paper, unless otherwise stated, have been conducted on uniformly infested soil near Granite City, Ill.

Plainly the results from this series of experiments do not show any striking influence of these substances upon the development of the disease.

In the fall of 1920 this work was modified, using smaller plots and adding fertilizer materials in somewhat greater quantities per acre. The plots in this experiment were 4 by 6 feet in size, consisting of uniformly infested soil. Harvest Queen (white-chaffed Red Cross) seed from a field free from the rosette disease was sown in each plot on October 11. The fertilizers and lime, except the acid phosphate, were applied just before seeding, the latter was applied just after seeding.

Table IV gives the treatments, the rates of application, and the extent of disease which developed in the plots.

As in the previous experiment, the results from this series show practically no influence upon the development of the disease. The slight variations in the extent of disease developing in the different plots during both seasons is easily within the limits of experimental error. In fact, such small differences are not apparent when the plots are observed casually.

In order to determine any possible influence of cropping methods upon the disease, a plot of infested land was sown to various crops in the spring of 1919. Another portion of the infested land was summer fallowed and still another portion of it was left in winter wheat which was badly infested with rosette disease. Table V gives the arrangement of these plots and the crops used. In the fall of 1919, after plowing and fitting, these plots were sown crosswise with a 54-inch drill strip of Harvest Queen (Salzer's Prize-taker) wheat.

TABLE V.—*Influence of previous crops and summer fallow upon the control of rosette disease on infested land during the spring of 1920, at Granite City, Ill.*

Crop grown in spring of 1919.	Crop grown in fall and spring of 1919–20.	Percentage of wheat plants showing disease in 1920.
Potatoes	Harvest Queen (Salzer's Prize-taker) wheat.	98
Rape	do	98
Winter wheat (affected by rosette).	do	94
Summer fallow	do	98
Buckwheat	do	97
Corn	do	93

In the spring the rosette disease developed with practically uniform severity on all the plots. Table V gives the relative percentage of the disease in each plot. While there was some variation in the percentage of disease occurring in these plots, the differences were very slight and the disease control was practically negative.

In the fall of 1920 Harvest Queen (white-chaffed Red Cross) seed was sown on infested land, part of which had been in summer fallow for two years and other parts in diseased and in healthy winter wheat the previous season. During the following spring the rosette disease developed in all the plots sown to the Harvest Queen (white-chaffed Red Cross) seed, and in no case was it possible to distinguish any difference in the percentage of disease present in the plots previously occupied by wheat and fallow. All contained from 90 to 95 per cent of diseased plants, showing conclusively that no control was obtained.

While many more fertilizers and combinations of treatments and more extended cropping measures could have been tested, it seems reasonable to believe that the experiments carried on covered a sufficiently large number of possibilities to warrant the drawing of rather definite conclusions therefrom.

While the extent of rosette disease was influenced very little in the various experiments just enumerated, the proportion of recovery after the development of the disease was somewhat less in the untreated than in the treated and fallowed plots.

In addition to the experiments on soil treatments and on rotation and tillage methods, soil disinfection experiments were carried on in connection with the work on parasitic factors. These experiments, which are described later, involved the treating of infested soils with a dilute formaldehyde solution previous to seeding in the fall. Such treatment resulted in a complete control of the disease.

While little investigation seems to have been made on the chemical reaction of formaldehyde in the soil, it appears to be rather generally believed that its action is confined largely to protein substances and that its chief effect in the soil is upon living organisms present.

The results of the experiments upon soil treatment and cropping methods and those obtained from soil disinfection, together with all the indirect evidence presented, seem to warrant the conclusion that improper nutrition or toxic substances, which may be in the soil as a result of continuous cropping or from the decomposition of organic or inorganic matter, are not to be looked upon as primary causes of the rosette disease.

PARASITIC FACTORS

This factor has been given more serious consideration than any other for the reason that a greater mass of indirect evidence points in its direction.

In order to throw some light upon this phase of the problem, it was decided to conduct experiments along lines of soil disinfection. It was reasonable to believe that if the disease is caused by certain types of parasites, soil disinfection should control the trouble. Two experiments were conducted on infested soil in the experimental plots located near Granite City, Ill.

The first experiment was conducted in six standard No. 00 galvanized steel garbage pails, 10½ inches in diameter by 11 inches deep. These pails were filled with heavily infested soil. The soil used in three of the pails was disinfected with a solution consisting of 1 part of 40 per cent formaldehyde and 49 parts water. This solution was sprinkled on thin layers of the infested soil as the soil was shoveled into the pails. Sufficient solution was used to produce a thick mud. This was done five weeks previous to sowing the seed. The three pails containing the untreated soil were handled in the same manner except that water alone was added to the soil. Pails were allowed to stand in the open to air and dry out for about five weeks. Harvest Queen (white-chaffed Red Cross) wheat, a susceptible variety, from a field free from rosette disease, was sown in each pail on October 8, 1920. The pails were set on a frame 1 foot from the ground and surrounded with sawdust held within a retaining wall (Pl. 5, A). This arrangement served to prevent unduly severe freezing and winterkilling. From 20 to 26 robust plants developed in each of the pails during the fall. No signs of the rosette disease developed during the fall period. In the spring, however, the disease devel-

oped in 92 per cent of the plants in the pails containing undisinfected soil. In the disinfected soil all plants were healthy and remained so during the season.[13] Plates 5, B, and 6 show the photographic evidence obtained from this experiment.

The second experiment consisted of two plots, A and B, each 4 by 6 feet in size, surrounded by wooden frames made of pine boards three-fourths of an inch in thickness and 8 inches wide. These frames were sunk in the soil 4 inches, leaving a 4-inch wall above the soil to keep out surface water (Pl. 5, A).

The soil inside these frames was heavily infested, like that used in the first experiment. In plot A, the dry surface soil was removed and a three-fourths-inch layer of the subsurface soil was removed and kept separate and not disinfected. The remaining soil was then removed from both frames to a depth of 10 inches, after which it was replaced in thin layers which were saturated with the formaldehyde solution previously described. Care was taken to return the soil layers in the same order as they were removed so that the original strata might be maintained as nearly as possible. After the disinfection the plots were allowed to air and dry for five weeks, after which the undisinfected layer of infested subsurface soil was returned to the surface and seed trenches of plot A. Then Harvest Queen (white-chaffed Red Cross) seed from a field free from the rosette disease was sown in rows 6 inches apart in both plots. Good germination was obtained and the resulting plants were very robust in the fall, showing no signs of rosette disease. In the spring essentially the same results were obtained as in the first experiment—that is, in plot A a high percentage of disease occurred, while plot B was essentially healthy. In the latter plot, however, a very few diseased plants developed around the edge, due doubtless either to splashing of water or seepage, or both, from the infested soil outside, indicating that the causal factor was disseminated to a slight extent in some such manner, apparently in the soil water. Apart from these few diseased plants, the plants in plot B were healthy and robust, remaining so throughout the season (Pl. 7, B). Plot A, which received the infested soil before seeding, developed the disease in about 75 per cent of the plants (Pl. 7, A).

Wheat growing in the infested soil just outside these plots developed from 85 to 95 per cent of rosette disease.

These results seem to prove conclusively that the rosette disease is caused by some factor other than winter conditions, soil type, or improper drainage; and, in view of the conclusions drawn in connection with soil factors other than parasitic, it seems that the evidence points most strongly in the direction of a causal organism or perhaps to some virus which may be greatly influenced by formaldehyde.

ANIMAL PARASITES

Insect parasites have been considered the cause of rosette disease by a number of investigators other than entomologists. A number of entomologists have examined plants affected by the rosette disease, and all have expressed the idea that insects are not the cause. In order to accumulate definite evidence on these points investigations have been conducted cooperatively by the writer and W. H. Larrimer of the Bureau of Entomology, United States Department of Agriculture. The results of these investigations will be published in a separate paper.

[13] Similar experiments carried on later with steam-sterilized soil gave the same results.

PLANT PARASITES

Close observations have been made for evidences of bacteria and fungi. While the first group has not yet been found consistently associated with the disease at any stage of its development, certain fungi have been found under certain conditions associated with the diseased plants in the later stages of their development, and also with the plants in the fall previous to the development of the rosette disease in the spring.

All of these parasitic forms, however, have been found associated to a greater or less extent with plants not showing rosette disease. Owing to the fact that the disease was not reported until rather late in the spring of 1919, investigations were not started until May of that year. Previous to starting the investigation and also afterwards, the pycnidia of *Septoria tritici* Desm. were found to be rather numerous on the leaves of many plants affected by rosette disease and also on the leaves of plants not showing the disease in question. This organism, however, was not consistently associated with all plants showing the rosette disease. In the spring of 1920 this organism was not often found in the field, probably on account of unfavorable winter conditions. During 1921 it was again prevalent in the experimental plots and elsewhere. Although this organism seems to be a leaf parasite primarily, the question of its possible parasitic relation to the roots and crown of the wheat plant is being investigated.

Upon starting the work in May, 1919,[14] it was found that a Helminthosporium of the *Helminthosporium sativum* type was almost always present within the tissues of diseased plants collected near Granite City, Ill., and the same organism was found associated to some extent with plants affected with the rosette disease from other parts of Illinois and from Indiana. On the other hand, the organism also was found rather prevalent upon plants free from the rosette disease. Many other forms of fungi were isolated from the basal tissues of plants showing the advanced stages of the rosette disease, but these were in the minority and less consistently associated with the diseased plants.

At that time of year (May) the presence of Helminthosporium in the tissues of plants affected by rosette disease was not considered especially significant, as this organism was not confined to plants showing the disease in question, or to the infested fields. Further, this organism had been known for a considerable time to be pathogenic on wheat, according to Johnson (*13*) and Bolley (*1*) and to be responsible for many of the wheat troubles in the winter- and spring-wheat belts, but these troubles differ in a number of respects from the rosette disease.

During the season of 1920 an intensive field study of the disease was made by the writer. Observations were started on March 24, just as wheat was coming out of the dormant condition. As soon as the disease made its appearance (March 26) platings were made from the basal tiller and root tissue of both diseased and healthy plants. This plating was conducted by the writer in the field laboratory located at Granite City, Ill., and by Mr. R. W. Goss, then with the Office of Cereal Investigations, located in the Department of Plant Pathology at the University of Wisconsin. Material used in the field laboratory was plated out within from one to two hours after the plants were taken from the soil. Material sent to the Wisconsin laboratories was collected just in time to make the

[14] Credit is due Mrs. Edith Seymour Jones for assistance in connection with the laboratory work done during the spring and summer of 1919.

best train connections and sent by special mail delivery. Ordinarily such material was in transit about 36 hours. The results of these platings have shown no organism to be consistently associated with the vital crown tissues of plants in the early stages of the development of the disease. By April 15 the same Helminthosporium which was found during the previous season made its appearance in the vital tissues of diseased plants and also on the tiller bases and the leaves of plants free from rosette disease. From this time on through the season this organism was found to be rather consistently associated with the disease. This was the period corresponding to the time when the work was started the previous year.

During the spring of 1921 a large number of platings were made from plants in all stages of the disease. Material was obtained from the experimental plots located near Granite City, Ill., and from the Indiana Agricultural Experiment Station plots located near Valparaiso, Ind. The plating was done by Dr. R. W. Webb in the laboratory of the Missouri Botanical Garden, at St. Louis, Mo., and by the writer in the laboratory of Plant Pathology at the University of Wisconsin. The results of these platings, which involved various media, reactions, and growing temperatures, were the same for the Granite City material as those obtained the previous year. In general, it may be stated that Helminthosporium was much less consistently associated with the diseased plants collected in Indiana than with plants collected near Granite City, Ill. The results of early platings were the same for material from both sources.

Experiments on the pathogenicity of pure cultures have been carried on with the predominating organisms isolated from diseased wheat plants. Of these organisms the Helminthosporium has been given the greatest attention. The results of this work show that this Helminthosporium is pathogenic on wheat, causing injuries which are apparently identical with those which heretofore have been produced by Johnson (*13*) with a similar or identical Helminthosporium isolated from wheat plants.

The types of injury produced by the Helminthosporium isolated from wheat plants in the late stages of rosette disease may be summarized as follows:

(1) Mild to severe infection of the coleoptile and first leaf of the germinating seed, often causing death of the seedling before emergence.

(2) Mild to severe root injury.

(3) Mild to severe infections of the underground leaf sheaths and tillers, from the seedling stage through the preboot and heading stage.

(4) Infection of leaves, causing characteristic lesions which may occur anywhere at the ligule or on the blade.

(5) Infection of leaf sheaths above ground, causing lesions very similar to those produced upon leaves; also infection of the nodes and internodes.

The same types of injury have been produced by the same type of Helminthosporium isolated from Wisconsin wheat not showing the symptoms of the rosette disease of wheat. Similar results also have been obtained by Stakman (*32*) with a Minnesota strain of Helminthosporium which appears to be very similar, if not identical, with the strains used by the writer.

While Stevens (*34*) claims to have proved that the rosette disease (called footrot by him) (*33*) is caused by Helminthosporium, it is not clear that his evidence warrants this conclusion. Careful examination made by the writer of Dr. Stevens' diseased plants from inoculation experiments [15] revealed only seedling and young-plant injuries identical with

those referred to above in connection with the writer's inoculations with Helminthosporium. It is entirely possible that Dr. Stevens restricts the name footrot to these limited types of plant injury, but if so the fact does not seem to have been made clear in his papers. Further, both in personal conferences and in public meetings subsequent to the publication of his second paper (*34*), Dr. Stevens has stated that he has obtained from inoculations with Helminthosporium only the seedling and leaf lesions and that he has not obtained from pure-culture inoculations the dwarfing and excessive tillering, the diagnostic characteristics of the rosette disease.

While experiments on pathogenicity conducted with the various organisms isolated from plants affected by the rosette disease have thus far failed to reproduce the characteristic symptoms of the disease, the writer does not feel justified in assuming that none of the organisms tested are the direct cause. As pointed out earlier in this paper, experience with the winter-wheat plant shows that the peculiar symptoms manifested by this disease do not develop readily under greenhouse

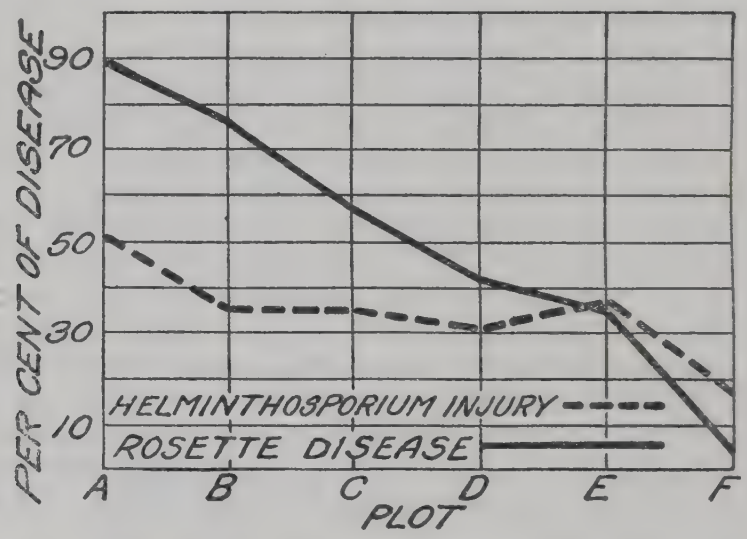

FIG. 2.—Graph showing tendency toward a correlation between the extent of rosette disease and the extent of Helminthosporium infection on wheat plants not showing the rosette disease in six experimental plots at Granite City, Ill.

conditions, and consequently further pathogenicity experiments are necessary before definite conclusions can be drawn.

A tendency toward a correlation between the percentage of Helminthosporium and of the rosette disease is shown in figure 2. The curves in this graph represent the percentage of both manifestations upon wheat plants growing in six date-of-seeding plots, the infestation of rosette disease in the soil being greatest in series A and rather gradually decreasing toward series F. The percentage of Helminthosporium injury in the various series was based on plants which escaped the rosette disease, thus enabling a direct comparison of results. On the whole the correlation between the two manifestations is rather striking and suggests a possible connection between Helminthosporium and rosette disease.

In following the development of Helminthosporium infection on wheat growing in the plots near Granite City, Ill., and elsewhere, it is found that infection takes place in the fall. Usually this infection obtains in the

[15] Exhibited at the Chicago meetings of the American Phytopathological Society in December, 1922

coleoptile tissues and in the outer underground leaf sheaths of the tillers. Sometimes a mild to severe infection takes place in the subcrown internode, but apparently this is not as common as the infection of the other tissues mentioned. Fall infection seems to be rarely severe, and in most cases tiller lesions do not penetrate beyond the first or second leaf sheath. Winter wheat sown early shows a higher percentage of Helminthosporium infection and also more severe injury therefrom than does late-sown wheat.

A close study of the healthy wheat plant late in the fall and through the winter indicates that practically all of the outer leaf sheaths disintegrate and become separated from the vital tissues of the tillers or in some cases disappear entirely. Similarly, in plants showing Helminthosporium infection on the outer leaf sheaths in the fall, these outer leaf sheaths disintegrate, leaving the inner living leaf sheaths practically free from lesions. This explains the comparative absence of Helminthosporium on and in the vital tissues of wheat plants early in the spring. In this connection soil-temperature experiments conducted under controlled conditions show that the minimum temperature for the development of the wheat plant is somewhat below that for the developement of Helminthosportium infection. This relationship probably explains why Helminthosporium does not begin its spring attack on the vital tissues of the plant until some little time after the wheat plant begins its spring development and also the increased fall infection of Helminthosporium in early sowings.

A condition somewhat similar to rosette disease has been noted by the writer on Marquis spring wheat growing in soil badly infested with Helminthosporium. Stakman (*32*) also reports a similar condition in spring wheat growing in Helminthosporium-infested soil in Minnesota. While this condition does resemble rosette disease in certain respects, it differs in others, as pointed out by Stakman.

Preliminary field studies made during the early winter of 1920–21 on Harvest Queen (white-chaffed Red Cross) wheat plants growing in soil known to produce the rosette disease showed that plants with fall infection of Helminthosporium on the subterranean portions of the tillers and on the subcrown internodes produce more tillers than plants free from such infection. Actual counts showed 42.5 per cent more tillers on the infected plants than on healthy ones.

In order to determine the influence of Helminthosporium upon tiller development, sterilized soil was inoculated in the greenhouse with a heavy water suspension of the conidia of the Helminthosporium isolated from a plant affected by rosette disease. Another plot identical with the former in every way except that the soil was not inoculated was used for the control. These plots contained about 10 square feet each. Harvest Queen (white-chaffed Red Cross) wheat (susceptible to rosette disease) and Marquis spring wheat (susceptibility questionable) were sown in both plots. The same number of seeds was used in each plot and for each variety, and seeding was done under the same conditions in each plot. The results of this experiment, given in Table VI, show a stimulation in tiller development in plants of the Harvest Queen (white-chaffed Red Cross) wheat infected with Helminthosporium, but not in Marquis.

While an increase in the number of tillers was obtained in Harvest Queen (white-chaffed Red Cross), this was noticed only by making

careful counts, as there was no striking indication of the characteristic stunting or blue-green color which occur on plants affected by rosette disease. Comparisons between Tables III and VI will show that while there is an increase in tiller development from the Helminthosporium inoculations, this increase is somewhat less than that found in plants showing rosette disease under field conditions.

While these are only preliminary data they indicate that Helminthosporium is capable of stimulating tiller development.[16]

TABLE VI.—*Influence of Helminthosporium infection upon tiller development in Marquis and Harvest Queen (white-chaffed Red Cross) wheat*

Variety.	Susceptibility to rosette disease.	Number of plants.		Reduction in stand on inoculated series.	Average number of tillers per plant.	
		Inoculated series.	Uninoculated series.		Inoculated series.	Uninoculated series.
				Per ct.		
Marquis	Questionable	240	263	8.7	2.66	2.65
Harvest Queen (white-chaffed Red Cross).	Positive	270	297	9.0	4.05	2.74

The correlations and other indirect evidences presented show that, under certain conditions at least, Helminthosporium is very closely associated with the rosette disease, and, as pointed out by the writer (*20*), there is a suggestion that the disease possibly may be an unusual manifestation of the Helminthosporium disease of wheat which has been known for some time to be present in several States (*1*), (*10*), (*32*), in the spring- and winter-wheat belts. Studies now under way will throw additional light upon the relation of this and other organisms to the development of the disease in question.

TOXINS AND VIRUSES

This group of factors has been considered among the possibilities of cause, and experiments now under way should throw some light upon it.

INTRACELLULAR BODIES

While it is possible that the intracellular bodies mentioned earlier in this paper may be some unusual type of organism which bears some relation to the cause of the rosette disease, it is also possible that they are reactionary bodies produced in the cells as a result of the disease.

Although these bodies have been found associated with the rosette disease, they have also been found associated with a mosaic-like leaf mottling in plants not showing the dwarfing characteristic of plants affected with the rosette disease. Thus far these intracellular bodies have not been found in wheat plants showing neither the rosette dwarfing nor mosaic-like leaf mottling. Further studies on both diseased and healthy plants are necessary before the nature of the bodies and their relation to the disorders can be definitely determined.

[16] Various workers, including Grantham (*5*) and Rimpau (*28*), have shown that tillering is stimulated by wide spacing between wheat plants. While the slight seedling killing in the inoculated plots resulted in a slight increased spacing, such spacing was practically the same in both varieties. Furthermore, no results have yet been found which indicate that such a small, irregularly distributed increase in spacing will increase tillering to the extent manifested in Harvest Queen (white-chaffed Red Cross) wheat.

SPREAD OF THE DISEASE

BY INFESTED SOIL

This point has been proved by experiments which involved the transferring of infested soil to small plots of disease-free soil located in Alhambra, Ill., and at Madison, Wis., and by introducing infested soil into soil sterilized with a 1 to 49 solution of 40 per cent formaldehyde and water at Granite City, Ill. The latter experiment is discussed under the head of parasitic factors.

In all cases the typical symptoms of the rosette disease developed in the plots located at the two points given and in the soil-disinfection experiment where undisinfected, infested soil was introduced. In every case healthy plants developed in the local and disinfected soil.

The summer-fallow experiments discussed under soil factors other than parasitic show that the causal factor can persist in the soil for at least two years without apparent loss of its disease-producing powers.

BY INFESTED SEED

In 1920 seed was selected from plants which had practically recovered from the attack of the rosette disease. In the fall this seed was sown in field plots located at Madison, Wis., and Alhambra, Ill., and also in disinfected soil at Granite City, Ill. While the resulting plants were not robust, due to the poor quality of the seed used, all plots and soil containers were free from any indications of the rosette disease. Adjacent plots receiving the infested soil in each case developed the disease.

While these results demonstrate that the disease was not borne by the seed used in the experiment it is not felt that they are conclusive. Certain field observations lead to the belief that under certain conditions the disease is seed-borne. The foregoing results tend to indicate that the disease is not due primarily to a "running out" nor to constitutional weakness of the susceptible variety.

BY INFESTED STUBBLE

Parallel experiments with the stubble of diseased plants were carried on with the seed and soil transmission experiments, and, as in the seed experiments, negative results were obtained. These results are not considered conclusive, however, and this point is being investigated further.

CONTROL MEASURES

Various control measures have been under investigation, and distinct progress has been made in controlling the disease through the use of resistant or immune varieties.

As noted previously, various experiments on the applications of fertilizers, manure, and lime, and involving limited cropping methods, have failed to effect a control of the disease, although most of these have increased the percentage of recovery after the disease has developed. Seed treatments also have failed to effect a control. This is to be expected, however, as the disease is soil-borne. The burning of stubble and the application of iron sulphate to the soil failed to give control. Extremely late seeding controls the disease to a considerable extent. However, the seeding must be late enough so that the plants do not emerge until the following spring, and for this reason the method is not practicable.

VARIETAL RESISTANCE

Extensive studies of varietal resistance have been made during the past two seasons. Many of the eastern wheat varieties have been grown on infested soil, and the disease has been controlled perfectly where resistant varieties have been used.

In 1919–20, 10 local varieties of wheat adapted to central Illinois were sown on uniformly infested land near Granite City, Illinois. The varieties were sown in adjacent parallel strips a drill-width (54 inches) wide and 50 rods long. The essential part of the writer's results from these experiments were first published by Reed and Dungan (*27*). Later the results were published by the writer[17] and by Humphrey, Johnson, and McKinney (*10*). These data are also given at this time in Table VII.

In 1920–21, 150 varieties and selections of winter wheat[18] were tested for susceptibility. Most of these were eastern varieties obtained from Dr. C. E. Leighty, of the Office of Cereal Investigations, but several were received from Mr. G. H. Dungan, of the Illinois Agricultural Experiment Station. All were sown by hand in rows 8 inches apart and 1 rod long, on uniformly infested land near Granite City, Ill. The varieties obtained from the Office of Cereal Investigations were sown in double rows and those received from the Illinois Agricultural Experiment Station were sown in single rows. A local susceptible variety, Harvest Queen (white-chaffed Red Cross), from a disease-free field was sown after every tenth variety throughout the series to serve as a control upon the uniformity of soil infestation. All control rows showed from 90 to 95 per cent of the rosette disease in the spring. Table VIII gives the list of varieties and selections and the percentage of disease developing in those which showed susceptibility. Owing to the limited size of these plots and to the fact that the stand in many of the plots was rather irregular, due to the very dry fall of 1920, yields were not taken on this series. Observations were made, however, on the general thrift of the varieties.

The outstanding feature of this experiment is the fact that only 6 per cent of all varieties showed susceptibility to the disease.

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[17] McKINNEY, H. H. RESISTANCE IN WHEAT TO THE SO-CALLED TAKE-ALL DISEASE. *In* U. S. Dept. Agr. Bur. Plant Indus. Off. Cer. Inves. Cereal Courier, v. 12, no. 17, p. 229–231. 1920. Mimeographed.

[18] In a recent publication on classification of American wheat varieties (CLARK, J. ALLEN, MARTIN, JOHN H., and BALL, CARLETON, R. CLASSIFICATION OF AMERICAN WHEAT VARIETIES. U. S. Dept. Agr. Bul. 1074, 238 p., 76 fig., 60 pl. 1922. Literature cited, p. 219–230), published since this paper was prepared, the following nomenclature has been proposed for certain of the varieties included in these experiments. In the following list the names used in this paper precede the corresponding ones used in the work cited: American Bronze= Prosperity; Budapest= Pesterboden; Crimean= Turkey; Dietz Longberry= Fulcaster; Early Harvest= Red May; Early May= Flint; Early Ripe= Red May; Enterprise= Red May; Eversole= Fulcaster; Ghirka Winter= Alton; Harvest King= Poole; Hedge Prolific= Poole; Hungarian= Pesterboden; Indiana Swamp= Valley; Lancaster= Mediterranean; Malakof= Turkey; Michigan Amber= Red May; Michigan Wonder= Red May; Miller's Pride= Mediterranean; Minnesota Reliable= Turkey; Missouri Bluestem= Mediterranean; Niagara= Gipsy; Orange= Red May; Pearl Prolific= Currell; Pennsylvania Bluestem= China; Pride of Indiana= Red May; Red Cross= Red May; Reliable= Gipsy; Stoner (Marvelous)= Fulcaster; Stoner= Fulcaster; Super (Burbank's)= Jones Fife; Theiss= Turkey; Velvet Chaff (Penquite)= Penquite; Winter Fife= Jones Fife; Worlds Champion= Turkey.

TABLE VII.—*Percentage of rosette disease infection, acre yield in bushels, and bushel weight of 10 varieties of winter wheat grown in plots 54 inches wide by 50 rods long, on uniformly infested soil, at Granite City, Ill., in 1920*

Variety.	Infection by rosette disease.	Acre yield.	Bushel weight.
		Bushels.	*Pounds.*
Harvest Queen ("Salzer's Prize-taker")	90 per cent	8.87	54.75
Red Wave	0	28.33	56.75
Illini Chief	25 to 30 per cent	24.08	58.00
Harvest King	Trace	28.83	56.50
Early May	0	25.61	58.50
Harvest Queen (white-chaffed Red Cross)	78 per cent	13.51	53.00
Fultz	2 per cent	26.20	59.50
Winter Fife	2 plants	32.87	58.50
Turkey	0	21.50	60.00
Fulcaster	2 per cent	25.80	60.75

TABLE VIII.—*Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921*

Variety.	Seed source.	Percentage of disease.
Susceptible:		
Harvest Queen (white-chaffed Red Cross)	Ill. Sta.	95
(Selection No. 13462, bearded, red-chaffed)	C. I. 4834	95
Harvest Queen	C. I. 4882	75
Niagara (Sel. No. 13535)	C. I. 5307	70
Velvet Chaff (Penquite)	C. I. 3540	65
Missouri Bluestem	C. I. 1912	60
Dawson (Dawson's Golden Chaff)	Ill. 9–225	Trace.
	C. I. 6161	Trace.
Illini Chief [1]	Ill. Sta.	Trace.
Illini Chief	C. I. 5406	Trace.
Budapest	C. I. 5789	Trace.
Turkey (Wis. No. 18) [1]	Ill. Sta.	Trace.
World's Champion [1]	do.	Trace.
Resistant:		
Ahrens	C. I. 4848	0
Alabama	C. I. 5785	0
American Bronze	C. I. 5638	0
Beloglina [1]	Ill. Sta.	0
	C. I. 5964	0
Crimean	C. I. 5831	0
Crimean × Fultz (R. 5, 1919) [1]	C. I.	0
Crimean × Fultz (R. 6, 1919)	C. I.	0
Crimean × Fultz (R. 7, 1919) [1]	C. I.	0
Crimean × Fultz (R. 8, 1919)	C. I.	0
Crimean × Fultz (R. 9, 1919) [1]	C. I.	0
	C. I.	0
Currell	C. I. 2906	0
	C. I. 3326	0
	C. I. 4802	0
Currell × Fultz (R. 12, 1919) [1]	C. I.	0
Currell × Fultz (R. 13, 1919)	C. I.	0
	C. I.	0
	C. I.	0
Dietz Longberry	C. I. 1981	0
	C. I. 3387	0
Early Harvest	C. I. 4582	0
	C. I. 4582	0

[1] Especially robust.

TABLE VIII.—*Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921*—Continued

Variety.	Seed source.	Percentage of disease.
Resistant—Continued.		
Early May	Local	0
Early Ripe	C. I. 5319	0
Enterprise	C. I. 3399	0
Evans	C. I. 2946	0
Eversole [1]	C. I. 3011	0
Fulcaster (R. 959, 1918)	C. I.	0
Fulcaster [1]	C. I. 3013	0
Fulcaster	C. I. 3407	0
	C. I. 4862	0
Fultz	C. I. 1923	0
	C. I. 3349	0
	C. I. 3423	0
	C. I. 3594	0
	C. I. 3598	0
Fultz type (selection from C. I. 3604)	C. I.	0
Fultz type (R. 408, 1918)	C. I.	0
Fultzo-Mediterranean	C. I. 3421	0
Ghirka Winter	C. I. 1438	0
Gipsy	C. I. 3459	0
	Ill. Sta.	0
	C. I. 3440	0
Gladden	C. I. 5644	0
	Ill. Sta.	0
Gold Coin	C. I. 5355	0
Gold Coin (Junior No. 6)	C. I.	0
Golden Wave	C. I. 6684	0
Grandprize	C. I. 5627	0
Harvest Queen (white-chaffed Red Cross, Salzer's Prizetaker).[1]	Resistant selection	0
Harvest King	C. I. 5647	0
Hedge Prolific	C. I. 4859	0
Hungarian [1]	Ill Sta	0
Indiana Swamp (white-chaffed) [1]	do	0
Jones Fife [1]	C. I. 5608	0
Jones Fife	C. I. 1942	0
Jones Paris Prize	C. I. 3568	0
Kanred [1]	C. I. 5146	0
	Ill. Sta.	0
Kharkof [1]	C. I. 5661	0
Lancaster	C. I. 1945	0
Leap	C. I. 5618	0
Malakof	C. I. 5663	0
Malakof [1]	Ill. Sta. No. 5–460	0
Mammoth Red	C. I. 2008	0
Mealy	C. I. 3563	0
	C. I. 5404	0
	C. I. 3565	0
Mediterranean	C. I. 3467	0
	C. I. 1395	0
	C. I. 3332	0
	Ill. Sta.	0
Michigan Amber [1]	C. I. 4864	0
Michigan Wonder	C. I. 5321	0
Miller's Pride	C. I. 4865	0
Minnesota Reliable	Ill. Sta.	0
New Amber Longberry	C. I. 3361	0
Nigger	C. I. 5652	0
Odessa	C. I. 6151	0

[1] Especially robust.

TABLE VIII.—*Percentage of rosette-disease infection in 150 varieties and strains of winter wheat grown in rod rows in uniformly infested soil near Granite City, Ill., in 1921*—Continued.

Variety.	Seed source.	Percentage of disease.
Resistant—Continued		
Ontario Wonder	C. I. 3843	0
Orange	C. I. 4868	0
Padui [1]	C. I. 6153	0
Palmer	C. I. 6685	0
Pearl Prolific	C. I. 3484	0
Pennsylvania Bluestem	C. I. 5342	0
Pesterboden [1]	Ill. Sta.	0
Poole	C. I. 5653	0
Portage	C. I. 5370	0
Pride of Indiana	C. I. 3492	0
Red Cross (red-chaffed)	C. I. 5318	0
	C. I. 3579	0
Red Hussar	Ill. Sta.	0
Red May	C. I. 5339	0
Red Rock	Ill. Sta.	0
Red Russian	C. I. 3497	0
Red Wave [1]	C. I. 5624	0
Red Wave	Ill. Sta.	0
Reliable	C. I. 3508	0
Rudy	C. I. 5625	0
(Selection) [1]	C. I. 2908	0
(Selection) (brown-bearded)	C. I. 3118	0
(Selection)	C. I. 3334	0
(Selection) (white-bearded)	C. I. 3135	0
(Selection)	C. I. 3068	0
	C. I. 4131	0
(Selection) [1]	C. I. 3554	0
(Selection)	C. I. 1593	0
(Selection from C. I. 3079)	C. I.	0
(Selection No. 131058)	C. I. 6685	0
(Selection No. 13838)	C. I.	0
(Selection No. 131218)	C. I.	0
(Selection No. 131156)	C. I.	0
Stoner (Marvelous)	C. I. 3605	0
	C. I. 5961	0
	C. I. 5961	0
Stoner	C. I. 2980	0
Super (Burbank's)	C. I. 5544	0
Theiss	C. I. 1561	0
Treadwell	C. I. 3527	0
Triumph	C. I. 3134	0
Trumbull [1]	C. I. 5657	0
Tule	C. I. 4140	0
Turkey	Ill. Sta. No. 514	0
Turkey [1]	Ill. Sta. No. 402	0
	Ill. Sta. No.	0
	Ill. Sta. No. 10–110	0
	Ill. Sta No. 12–41	0
Turkey	Ill. Sta. No. 509	0
Turkey (Wis. Ped. No. 2)	Ill. Sta.	0
	Wis. Sta.	0
Turkey (Iowa No. 404) [1]	C. I. 5580	0
Turkey [1]	C. I. 6152	0
Turkish Amber×Dale Gloria (R. 3, 1918)	C. I.	0
Turkish Amber×Dale Gloria (R. 4, 1919)	C. I.	0
Wheedling	C. I. 4846	0
Winter Chief	C. I. 4878	0

[1] Especially robust.

A few of the varieties which showed susceptibility in 1920 did not show the disease in 1921. This occurred only among varieties showing low percentages of the disease in 1920. It is possible that the varieties showing the small percentage of infection were mixtures, while in those varieties showing high variations in the percentages of disease and nonsusceptibility a genetic relation existed. This latter conclusion is based upon results obtained in selections studied in 1920–21 by the writer.

In all the experiments it has been found that certain plants within the Harvest Queen (Salzer's Prizetaker) variety (susceptible) always survived or escaped the rosette disease and that such plants showed the same general agronomic characters as the varietal type. At first it was thought that these were accidental escapes, but upon selecting the heads from such plants and sowing the seed from these on infested land (Pl. 8, A, *a*) 100 per cent resistence was obtained in all resulting plants and the head and kernel type remained true.

Further experiments are necessary to determine the resistance and susceptibility of additional varieties and to complete the study of many of the varieties and selections already tested.

It is evident from the results of the trials that the Harvest Queen (Salzer's Prizetaker and white-chaffed Red Cross), Illini Chief, Velvet Chaff, Missouri Bluestem, Niagara, and Indiana Swamp varieties are very susceptible to the disease and should not be grown in any of the districts where the rosette disease occurs.

The variety grown in central Illinois under the name Salzer's Prizetaker has white chaff and red kernels, and it is this variety which is susceptible. The original Salzer's Prizetaker was a variety with brown chaff and white kernels. This latter variety is identical with Gold Coin and Gold Coin (Junior No. 6), as listed in Table VIII, which have been found immune from the disease.

The susceptible variety Harvest Queen, known locally as Red Cross in central Illinois, also has white chaff and red kernels and is identical with the susceptible Salzer's Prizetaker variety. True Red Cross has red chaff and is a distinct variety grown to some extent in the eastern United States under the name of Red Cross. This variety was tested in 1921 and found to be resistant to rosette disease. All these Salzer's Prizetaker and Red Cross varieties are beardless. The variety known as Harvest Queen is supposed to be the same as the susceptible white-chaffed "Red Cross" variety. Harvest Queen is grown in the Missouri River Valley in eastern Kansas.

Certain of the resistant varieties are extensively grown in the infested area in Madison County, Ill., and the writer has made rather extensive observations on such varieties growing under farm conditions. Varieties which have been observed to maintain their resistance and show indications of immunity are Early May, Red Wave, and Jones Fife. While all of these are good yielding varieties in central Illinois, it is doubtful if they come quite up to Harvest Queen (Salzer's Prizetaker and white-chaffed Red Cross) in all respects. The latter variety is a favorite among farmers who do not have the rosette disease on their farms. This variety stands the winter well, is very well adapted to the less fertile soils, yields well, and produces grain of very high quality. It is hoped that the resistant selections which have been made from Harvest Queen (white-chaffed Red Cross and Salzer's Prizetaker) will retain all of the good qualities found in the variety and that the resistance to the rosette disease will be found to be stable.

The outstanding result of the variety tests is that the great majority of winter wheat varieties are resistant to or immune from rosette. While this ratio between resistant or immune and susceptible varieties is unusual, it is paralleled in the cases of the Lahaina, Sereh, and Fiji diseases of sugar cane.

INFLUENCE OF SEEDING DATE

In order that information might be obtained on this phase of the problem, a series of sowings was made during the fall of 1919 on land which developed uniform rosette infestation during the previous season. Two susceptible strains of Harvest Queen wheat (Salzer's Prizetaker and white-chaffed Red Cross) were used in the experiment. Each variety was sown on five different dates, except that, through error, Early May wheat was sown in the place of the first sowing of the white-chaffed Red Cross.

Each sowing consisted of a single strip 54 inches in width and 6 rods long. All seed was sown with an 8-spout disk drill. Seed was treated with formaldehyde and sown at the rate of 6 pecks per acre on September 18 and 24, October 4 and 16, and November 18. The results are given in Table IX.

TABLE IX.—*Percentage of rosette disease developed in strains of Harvest Queen wheat (Salzer's Prizetaker and white-chaffed Red Cross) sown in plots 54 inches by 6 rods, on five different dates in 1919, at Granite City, Ill.*

Strain of Harvest Queen wheat.	Percentage of disease in plots seeded on different dates.				
	Sept. 18.	Sept. 24.	Oct. 4.	Oct. 16.	Nov. 24.
Salzer's Prizetaker	95	90	90	79.0	4.5
White-chaffed Red Cross	No sowing.	78	78	73.8	0.28

In the first four sowings there was normal fall emergence, but from the sowing of November 18 emergence did not take place until spring. In the first sowings there was a little Hessian-fly injury in the fall. In the October 16 sowing considerable winterkilling took place. Both of these complications made it necessary to calculate the amount of Hessian-fly and frost injury in other neighboring plots not affected by the rosette disease in order that corrections might be made in the rosette disease plots.

Except in the November 18 sowing, a high percentage of disease developed in all the plots. Reference to Table X will give the exact percentage of the disease in each plot after corrections have been made for injury by Hessian fly and frost. It is quite evident that the date of seeding influences the development of the rosette disease, but this relation seems not to be of such nature as to permit economic application. It is remarkable that the disease should be so slight in the sowing that did not emerge until spring (Pl. 8, B).

The result of this field experiment shows very conclusively, on the basis of the November 18 sowing, that the problem is largely a seasonal one, and it bears out the general contention that the disease can not be produced successfully in a high percentage of plants under ordinary greenhouse conditions or in the field out of the regular season for the winter wheat plant.

Plate 8, B, shows the November 18 sowing as it appeared on May 21, 1920. While very little tillering took place on the plants in this plot, a very good quality of wheat was produced at the rate of 22 bushels per acre, with a bushel weight of 60.75 pounds. From the agronomic standpoint, this is remarkable for winter wheat sown on such a late date. For spring-sown winter wheat no such result is obtained. The resulting plants tiller vigorously, without shooting or heading, and produce no crop. The physiological basis for the behavior of the November 18 sowing affords a very interesting problem. Doubtless the effect of soil temperatures in relation to the stages of plant development is largely responsible.

The date-of-seeding experiment was repeated in the fall of 1920, using Harvest Queen (white-chaffed Red Cross) seed. The plots were the same width as those used the previous year, but they were only 2 rods long. Sowings were made on September 21, October 4 and 11, and November 4 and 19.

TABLE X.—*Influence of seeding date upon the percentage of rosette disease developed in Harvest Queen (white-chaffed Red Cross) wheat sown in plots 54 inches by 2 rods on five different dates in 1920*

Date of seeding	Sept. 21.	Oct. 4.	Oct. 11.	Nov. 4.	Nov. 19.
Percentage of disease	93.6	85.6	96.0	36.6	39.4

In connection with this date-of-seeding series it should be noted that owing to the mild winter the last two sowings made in November, 1920, emerged between Christmas and New Year's and a good stand resulted in the early spring.

The results from this series are shown in Table X. While a considerable percentage of rosette disease developed in the two latest sowings, it should be noted that the symptoms differed considerably from those manifested by plants in the early sown plots where normal fall emergence took place. The disease did not appear until fully six weeks after it had developed in the early sown plots. At this time the plants were from 12 to 16 inches in height. The first indications consisted in a retarding of certain plants followed by the dark blue-green coloration found in typical plants. Only slight excessive tillering resulted, and but few diseased plants developed normal heads. These same modifications in symptoms take place under conditions of reduced temperature in the greenhouse.

Just why a greater percentage of disease developed in the last two sowings made in 1920 than in the last sowing made the year previous can not be explained satisfactorily at this time unless it is for the reason that a steadier growth followed emergence in the sowing made on November 18, 1919, than with the last two sowings made in 1920. The latter sowings were subjected to a rather limited dormant period after emergence, thus simulating more nearly the conditions surrounding early fall-sown wheat.

It will be noted from Table X that there was an increase in the percentage of disease which developed in the sowing of October 11, 1920, compared with the two earlier sowings, thus causing a decided rise in the curve. It will be noted also from Table IX that there was a constant decrease in the percentage of disease in the 1919 plots from successively

later sowings. No exact explanation of this irregularity in the disease curves in the two seasons can be given, but it is noted that these curves follow closely the temperature curves for the germination periods for the several sowings. This relationship is very suggestive of a parasitic cause.

SUMMARY

This paper deals with the rosette disease of winter wheat, which was first reported in 1919 from Madison County, Ill., and a little later from Indiana under the name of take-all and later so-called take-all.

Investigations show that this disease is not the same as the take-all and footrot type of diseases which have occurred for many years in Australasia and Europe and have now been found in the United States.

The rosette disease has recurred in the field each year since it was reported, but the typical plant symptoms have not been reproduced satisfactorily under ordinary greenhouse conditions or in the field out of the regular season for winter wheat, thus reducing the problem to a seasonal one until such time as special methods have been perfected.

The origin of the rosette disease of wheat is unknown. It is believed that the disease has been present in certain of the infested areas for a considerable length of time.

So far as known this malady occurs only in the States of Illinois and Indiana.

Under conditions favorable for the disease it may be very destructive, causing a reduction of 75 per cent or more in yield in experimental plots. Under conditions favorable for the host, remarkable recovery from the disease may result and losses may be very slight. Forty per cent reduction in yield is not uncommon under farm conditions.

So far as definitely known the disease occurs only in certain varieties of winter wheat. Certain symptoms resembling the rosette disease have been observed in certain varieties of spring wheat and to a slight extent in barley and rye. No other grains or crops under experiment have developed symptoms resembling the disease.

The disease may cause a spotting in affected fields or it may be distributed rather generally over the fields. These spots or patches of diseased plants occur independent of any particular soil type or topographic condition in the field.

The most constant or characteristic plant symptoms consist of: (1) an arrested spring development, (2) an excessive tillering which results in a rosette appearance, and (3) a dark blue-green color of the foliage in combination with 1 and 2.

The take-all and footrot type of diseases cause field spotting, which is similar to the spotting caused by the rosette disease, except that the former diseases seem to appear a little later in the spring.

In the take-all and footrot type of diseases no excessive tillering has been noted, and diseased plants turn yellow shortly after the spring growth begins. Such plants gradually bleach and dry up, usually standing erect and assuming a stiff, wiry posture. Sometimes older plants break over at the base. On the other hand, plants affected by the rosette disease, when killed early, form a flat or drooping tuft of brown dead leaves which do not pass through a yellow stage.

A black "mycelial plate" is rather characteristic of the take-all and footrot type of malady, but this has never been found associated with the rosette disease.

Premature ripening or the development of "white heads" is commonly found in take-all and footrot, but in the rosette disease, delayed ripening occurs in those plants which partially recover from the disease.

The take-all and footrot type of trouble occurs in varieties of wheat known to resist the rosette disease.

None of the fungi commonly associated with take-all and similar footrots have been found associated with the rosette disease.

The rosette disease is diagnosed with greatest certainty in the spring before healthy plants reach the boot stage.

The rosette disease behaves in many ways similar to the Fiji, Sereh, and mosaic diseases of sugar cane and the mosaic disease of corn.

The cause of the disease is unknown.

Soil disinfection experiments made in the field show that a solution of 40 per cent formaldehyde and water (1 to 49) applied to infested soil will control the disease. The disease developed in nondisinfected plots of the same soil located directly adjacent to the disinfected soil. The same seed, Harvest Queen (white-chaffed Red Cross), was used in both cases.

A similar experiment conducted using steam-sterilized soil gave the same results.

The foregoing experiment together with winter temperature records proves that winter injury and temperature are not the prime causes for the rosette disease. This evidence, together with that obtained from fertilizer and fallowing experiments, indicates that nonparasitic soil factors probably are not the cause of the disease.

The results from soil disinfection point most forcibly to an organism or perhaps a virus as being the causal factor of the disease.

There is no indication that insects are the cause of the trouble. This view is maintained by a number of prominent entomologists.

Certain unusual intracellular bodies have been found associated with wheat plants showing the very early stages of rosette.

A number of fungi have been found associated with diseased plants during certain stages, but none has been found consistently associated with the trouble when it makes its first appearance in the spring.

It has been proved that the disease is soil-borne. While experiments have not shown that the disease is seed-borne, certain field observations indicate that it possibly may be.

The causal agent of the disease is known to persist in summer-fallowed soil for at least two years without apparent loss of its disease-producing powers.

The disease is controlled through the use of resistant varieties.

Only 6 per cent of the 150 varieties and selections used in experiments have shown definite susceptibility.

Extremely late fall seeding with spring emergence of seedlings practically controls the disease, but this method of control is not practicable.

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PLATE 1

Healthy and diseased Harvest Queen wheat plants of same age.

A.—Healthy plant showing good development and normal green color.

B.—Plant showing early symptoms of rosette disease, namely, marked dwarfing, excessive tillering, blue-green color, and some leaf mottling before Helminthosporium invasion has started.

C.—Plant attacked by rosette disease, showing symptoms after Helminthosporium rot has developed. General symptoms similar to those in B, but with marked rotting and discoloration of basal portion.

A
B
C

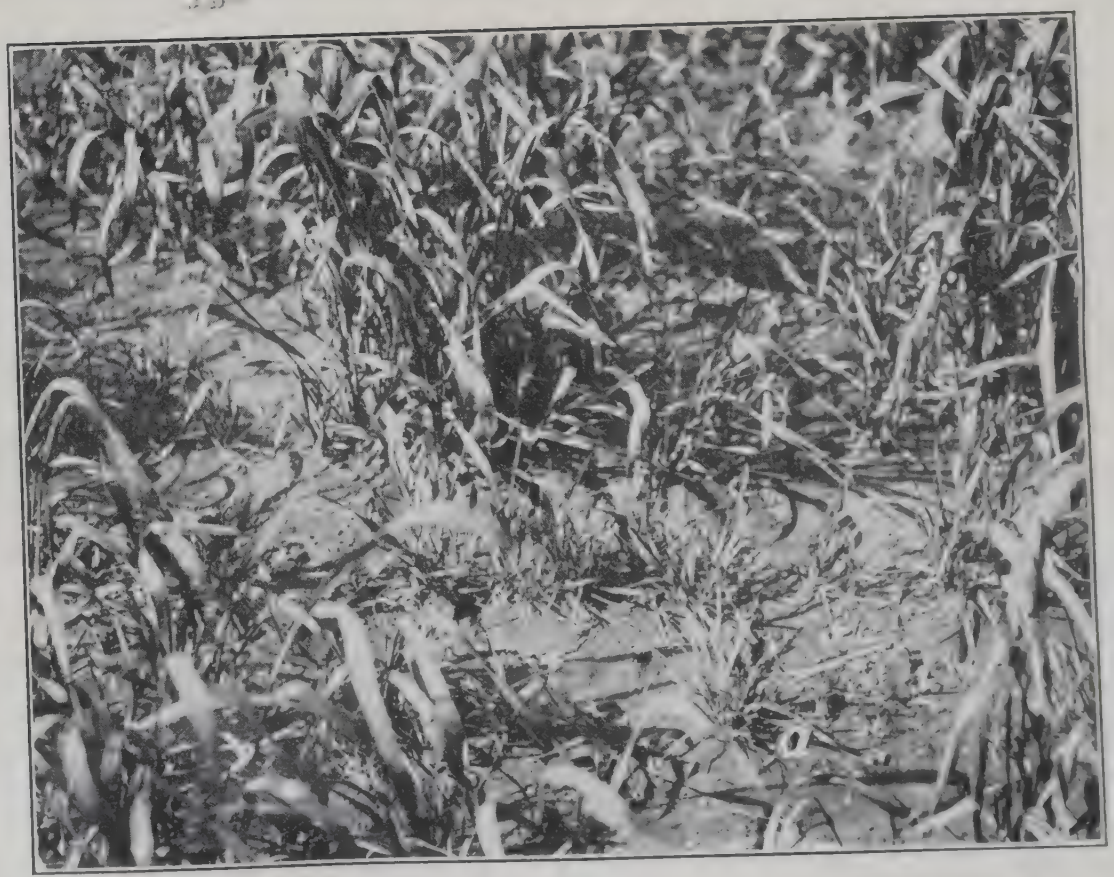

PLATE 2

Spot in Field of Harvest Queen (Salzer's Prizetaker) Wheat Caused by the Rosette disease. Diseased plants (*a*) are greatly dwarfed in contrast with surrounding healthy ones.

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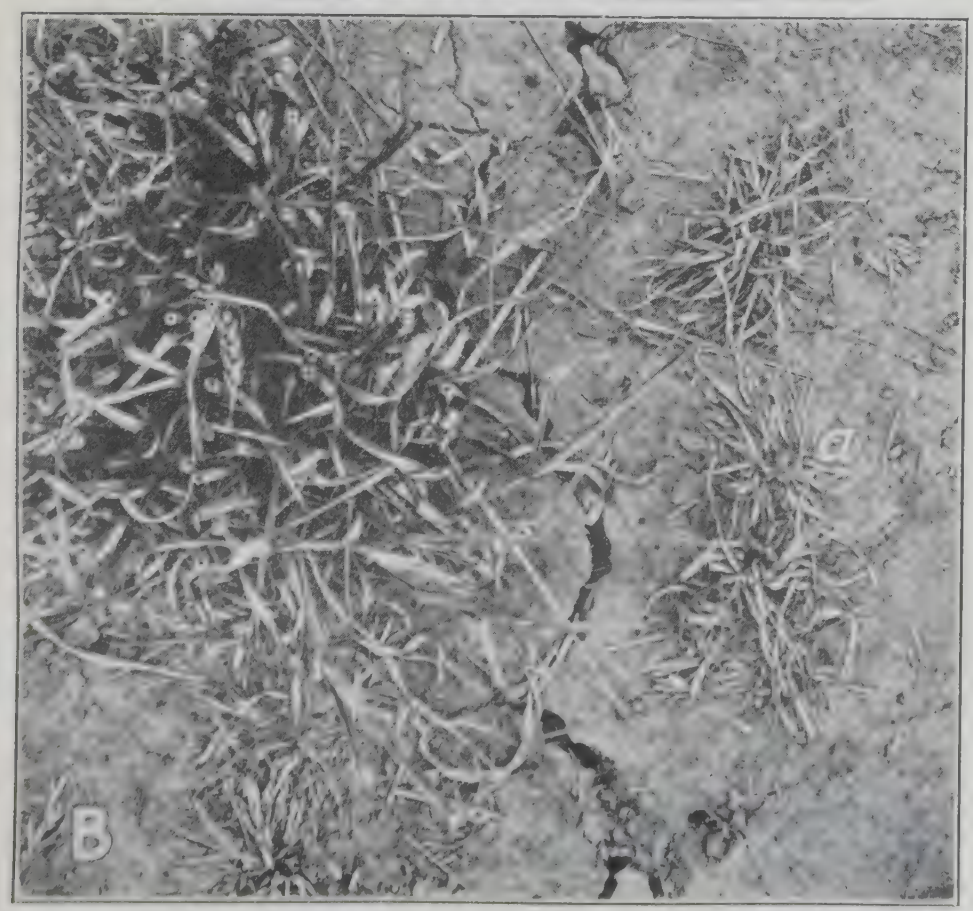
PLATE 3

Single wheat plant (A,*a*) attacked by the rosette disease among surrounding healthy ones, and bird's-eye view at harvest time of remains of wheat plants (B,*a*) killed by the rosette disease early in the season, with stubble of healthy plant at left.

A
a


B
a

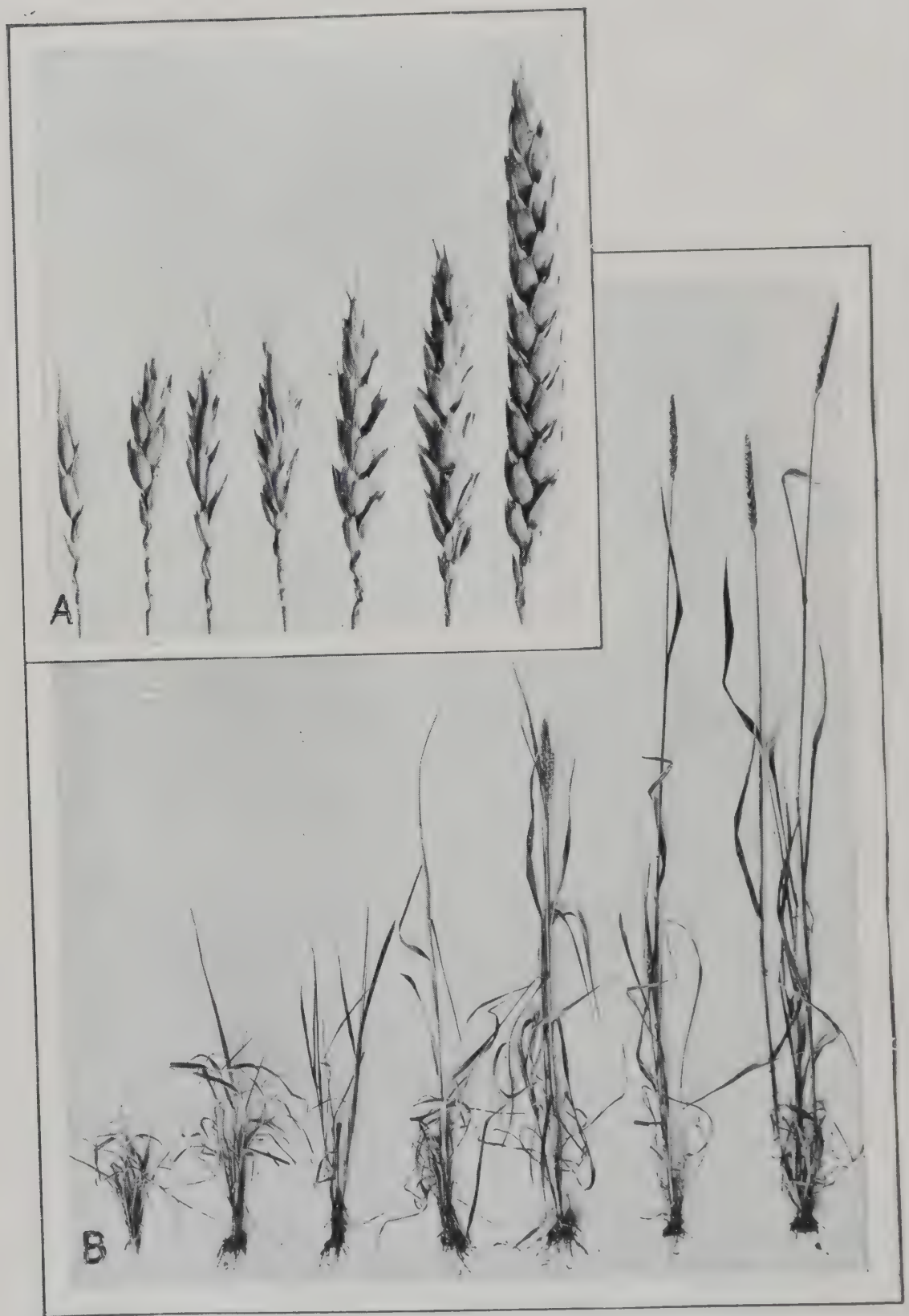

A
B

PLATE 4

Heads and plants of Harvest Queen (Salzer's Prizetaker), diseased and healthy.

A.—Six poorly developed heads (left) from plants partially recovered from the rosette disease, and one typical healthy head at (right).

B.—Six plants (left), all of same age, showing various degrees of recovery from the rosette disease. Healthy plant at right.

PLATE 5

A.—General view showing boxed-in soil plots in foreground and at right and galvanized-iron pail series in background at left.

B.—At left, two pails containing healthy plants of Harvest Queen (Red Cross) wheat grown in infested soil that had been disinfected with formaldehyde previous to sowing the seed. At right, two pails containing plants of Harvest Queen wheat of same age grown in infested soil undisinfected. Healthy seed used throughout. Typical rosette disease developed only in the undisinfected soil.

A

B

A
B

PLATE 6

Plants of Harvest Queen (Red Cross) wheat from disinfected (left) and undisinfected (right) pails of soil.

A.—Healthy plants from one pail of disinfected infested soil shown in Plate 5, B at left.

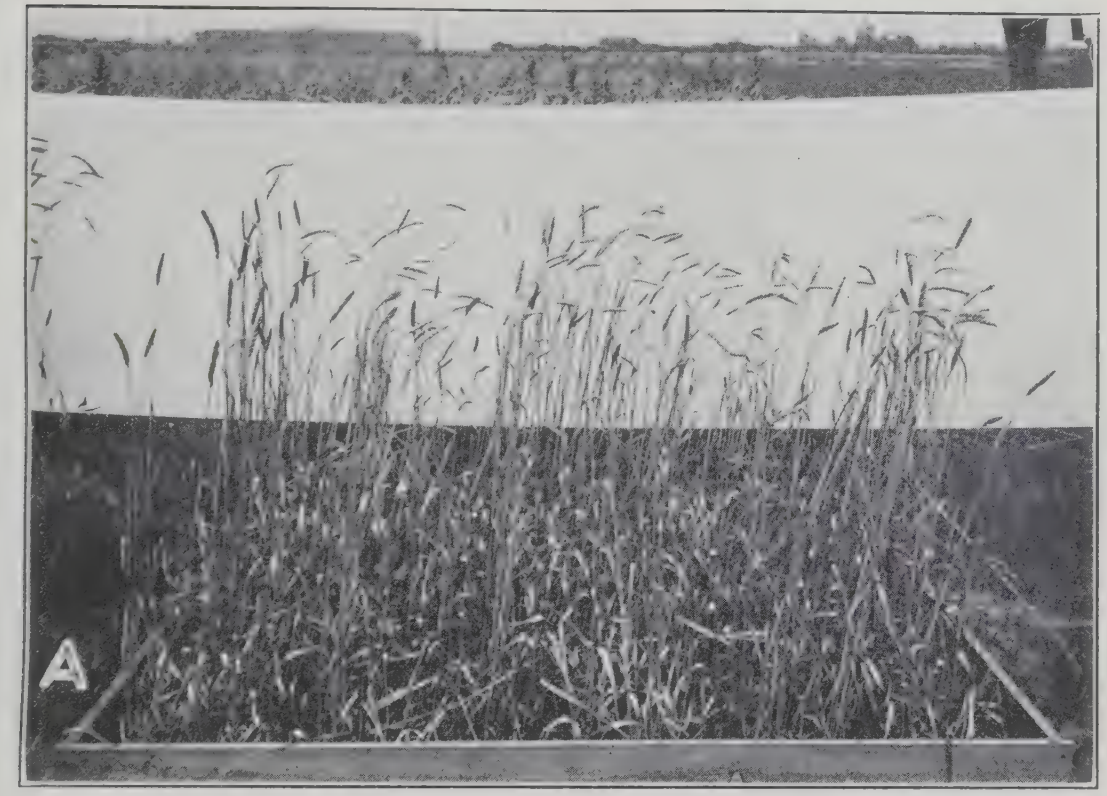
B.—Diseased plants from one pail of undisinfected infested soil shown in Plate 5, B, at right, showing severe attack of rosette disease.

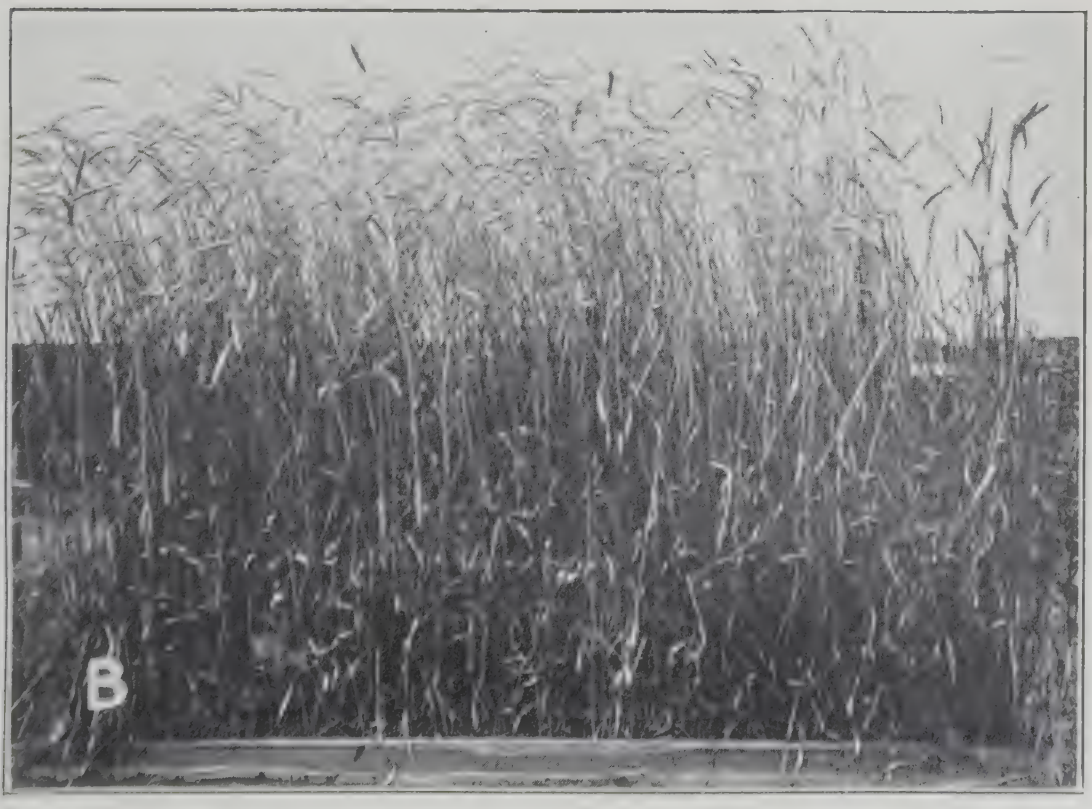
PLATE 7

Plants of Harvest Queen (Red Cross) wheat grown in plots of inoculated (A) and uninoculated (B) disinfected soil.

A.—Thin stand caused by severe attack of rosette disease. The soil in this plot had been disinfected in the same way as that in B, but was inoculated with a small amount of infested soil when seed was sown.

B.—Good stand of wheat. The soil in this plot had been disinfected with formalhyde previous to seeding and was not inoculated. Both A and B were sown at same rate and at same time with clean seed.


A


B

A
a
b

a
b
B

PLATE 8

The control of the rosette disease by the use of resistant varieties and by very late fall planting.

A.—*a*, Healthy wheat plants grown from specially selected seed from resistant plants of Harvest Queen (Salzer's Prizetaker) grown on infested land; *b*, diseased wheat of same age and of same variety grown from unselected seed. Plot *b* showed 90 to 95 per cent of rosette disease.

B.—*a*, Good stand obtained on uniformly infested soil by very late fall sowing. This plot was sown November 18, 1919, and plants did not emerge until the following spring; *b*, thin stand of dwarfed wheat, heavily infected with the rosette disease. This plot was sown October 16, 1919, and the plants emerged in the fall. The thin stand is due in part to winter injury, but the dwarfing is caused by the rosette disease.

ACCUMULATION OF ALUMINUM AND IRON COMPOUNDS IN CORN PLANTS AND ITS PROBABLE RELATION TO ROOTROTS [1]

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INTRODUCTION

The rots of the roots and stalk parts of the corn plant present a very complex problem, the entities of which are difficult to isolate for study and interpretation. Certain fungi and bacteria, which are more or less common in all fields in which corn is grown commercially, are almost always present in these rotted tissues. At times the rot diseases may be very severe, but the damage caused by them seems to be influenced by certain soil conditions which are associated with deficiencies of the essential nutrients or with unbalanced combinations of available salts for absorption by the plants. Frequently the extent of damage may vary markedly in certain parts of fields or in different fields wherein the same seed stock had been planted.

One of the most common characteristics of root rotted plants and of plants growing in certain areas in some fields is the brown to brownish purple discoloration of the vascular plate tissues of the nodes, as shown in Plates 2 and 5. This nodal discoloration has been observed in plants in all stages of development. It has been found in plants growing in all parts of the Corn Belt.

The most common difference between plants which show indications of being diseased and those which are of normal growth and bear normally matured ears is this almost universal brownish purple discolored condition of the nodal tissues. Attempts to isolate organisms from these tissues frequently prove futile, although in a large majority of cases the common *Fusarium moniliforme* Shel., *Gibberella saubinetii* (Mont.) Sacc., a white bacterium, *Penicillium* sp., *Rhizopus* sp., and others are found to be present, especially if the nodal tissues in the lower parts of the stalk are cultured.

This nodal discoloration has been found in young plants which show no rot lesions whatever on the roots and from which no organisms could be isolated. Later in the season, however, abundant rootrots have developed in the plants, especially in "spots" in the fields where the majority of the plants showed these nodal discolorations, and much damage resulted. Field observations and experiments show that the largest numbers of seedling injuries also occur in the soils where these

[1] Accepted for publication July 23, 1921. The results reported in this paper have been obtained in investigations conducted jointly by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and Purdue University Agricultural Experiment Station.

nodal troubles are most abundant. The types of soil which are high in organic matter and low in calcium and phosphorus seem to be the best environment for the development of seedling-blight. Because of these relations an attempt was made to ascertain the cause of the brownish purple discolorations of the vascular plate. Microchemical tests made upon these discolored tissues proved the presence of organic compounds of iron and aluminum in them.

The purpose of this paper is to discuss the methods used to detect these metallic accumulations and the methods used to determine the cause of the nodal discolorations and subsequent disintegrations and their probable importance in relation to the development of the rootrots, stalkrots, and earrots of corn.

OCCURRENCE OF IRON AND ALUMINUM IN PLANTS

Iron compounds are essential to the normal development of all green plants. Their importance has been demonstrated by numerous experiments, the history of which needs no reference here. Chlorosis, which has been directly referable to conditions which develop in the plant and which is associated with deficient quantities of available iron salts, is well known and its causes are more or less understood.

Less is known regarding the occurrence of aluminum in plants, although considerable work has been done in the study of its rôle in the soil as a factor affecting the growth of plants, both as a stimulant and as a toxic agent.

Mazé (*17*)[2] believes that the presence of aluminum in the soil is necessary for the normal development of maize. He also includes iodin, fluorin, and boron as being necessary. True, Black, and Kelley (*25*) report a higher aluminum content of the tops of spinach when the plants are diseased than when they are healthy. Kratzmann (*14*) reports the ash content of the leaves of maize to contain 2.35 per cent of aluminum. A study of 130 different plants showed that aluminum is widely distributed, and the unusually large quantities present in some plants suggested that certain species may be "aluminophilous." According to Kratzmann, different plants possess a specific elective power toward aluminum. Two plants of different species may accumulate widely different quantities of aluminum even though grown close together.

This same phenomenon of varying capacities for the absorption of aluminum by different plants seems to apply equally well to different individuals within a definite variety of corn, or even to individual plants grown from different kernels from the same seed ear. These differences may be interpreted as being due to the heterozygosity of the strain of corn. Qualitative tests for iron and aluminum applied to many plants studied in the rootrot investigations show very variable amounts of iron and aluminum in different stalks. From these observations it is evident that Kratzmann's designation of specific elective powers of plants for aluminum applies to corn for both aluminum and iron. The conditions which develop in the corn plant when large quantities of iron and aluminum salts enter the plant and concentrate in certain tissues have not been studied so far as can be learned.

[2] Reference is made by number (italic) to "Literature cited," pp. 822–823.

SELECTIVE ABSORPTION IN RELATION TO RESISTANCE TO ROOTROTS

It is believed that resistance of the corn plants to rootrots may be closely related to what appears to be a selective absorptive capacity of plants toward aluminum and iron when these metals are available in subtoxic concentrations. Selective absorption may be a very important heritable character in corn. If so, the phenomenon of susceptibility to rootrots which is associated with the accumulation of abundant quantities of iron and aluminum in different parts of corn plants may have as one of its causes a definite type of selective absorption for aluminum and iron salts. Therefore, the accumulations will occur in the plants under conditions in the soil when the quantities of these available salts are in subtoxic proportions. Such conditions usually are found in most acid soils.

It is true also that the quantity of available aluminum compounds in some soils may be sufficient to more than overcome the natural resistance possessed by strains which ordinarily show good growth in soils with lower concentrations of available iron and aluminum salts. In this case, irrespective of strain differences, the plants may be severely affected and a distinct toxic effect of the aluminum be noted in all of them. Plate 4 shows two fields where many of the plants were severely stunted. All of these plants had the typical nodal discolorations and disintegrations of the basal tissues shown in Plate 2, B.

A field of Stowell's Evergreen Sweetcorn was seen at Decatur, Ill., in August, 1920, in which all the plants were stunted in that part of the field which had been in clover sod the preceding season. In the part of the field which had been in corn the plants showed good growth. The plants which were stunted showed concentrations of the metals in the nodal tissues, but no root lesions were noticed at that time. Later in the season many of these plants became severely rotted. The plants which had made better growth on the corn land were notably less seriously attacked. The soil was the typical Illinois black loam and gave an acidity reaction of $P_H 5.6$ for the clover-sod land and $P_H 6.2$ for the corn land.

The nodal-tissue injuries shown in Plate 2, B, and Plate 5 are not the only effects resulting from the accumulation of these metals in the cornstalks. Analyses of the various plant parts show that the metals are distributed throughout the plant. The very obvious effects of the metals upon the nodal tissues gives them a seemingly greater importance in these tissues, but the unbalanced absorption of essential nutrients which takes place when these discolorations of the tissues are produced also accounts for a disturbance in the normal functioning of other active tissues.

Analyses of the leaves from diseased and healthy stalks show wide variations in the quantities of aluminum in them. The average aluminum (Al_2O_3) content of the ash of the leaves of four selected normal stalks from a field near Battleground, Ind., in 1920, was 0.44 per cent, while the aluminum content of the ash of the diseased plant leaves was 0.98 per cent, over twice as much. The calcium (CaO) content was 0.70 per cent in the normal leaves and 0.56 per cent in the leaves from diseased stalks.

The percentage of aluminum in the ash of the leaves from two root-rotted stalks from Bloomington, Ill., in 1918, was 1.16 per cent, while that of the leaves from stalks which remained healthy, and which were grown from the same seed ear, was 0.67 per cent. These cases which have just

been cited suggest that the influence of the aluminum salts may be harmful in the leaves and that, while the stalks which are diseased seem to have large quantities accumulated in the nodal tissues, these accumulations may indicate only the relative quantities which have been carried on to the leaf tissues to concentrate there and become toxic when in sufficient quantity. The problem of chlorophyll disturbances is a most complicated one, and the influence of these metals as catalysts upon the production of chlorophyll and as harmful agents upon the functioning of the chlorophyll tissues is little understood. The metals affect the chlorophyll tissues differently according to the amounts of alkaline bases present in the leaves.

AVAILABLE ALUMINUM SALTS IN SOILS

Much has been written of late relative to available aluminum and iron salts in the soil and their relation to soil acidity. Extracts from some acid soils have yielded quantities of aluminum salts in toxic concentrations. One of the explanations of the phenomenon of acidity in the soil is that it arises from the hydrolysis of salts of iron and aluminum.

In 1913, Abbott, Conner and Smalley (*1*) reported the results of their investigations upon an unproductive acid soil in northern Indiana. They found aluminum nitrate present in the soil extracts and attributed the acidity of the soil to the hydrolysis of the aluminum nitrate. Daikuhara (*8*) believes that salts of iron and aluminum are absorbed by the humic acids and other colloids of the soil and are set free again on treatment with neutral salt solutions. He infers that such compounds must exercise considerable influence not only on the acidity of purely mineral soils but also on that of soils containing humus. The injury to vegetation is thought to occur when the compounds of iron and aluminum absorbed by the soil colloids are liberated as soluble acid salts of iron and aluminum upon the application of mineral fertilizers.

In 1916, Conner (*5*) stated that much of the harmful acidity of acid soils is due to the presence of toxic acid salts of aluminum and iron and that the reduction of soil acidity by acid phosphate probably is due principally to a combination of the soluble phosphoric acid with the acid salts of aluminum and the consequent formation of insoluble nonacid compounds.

Spurway (*22*) attributes the acidity of certain glacial soils to the "hydrolytic-ratio" between the salts of the alkaline earth metals on the one hand and those of iron and aluminum on the other.

Hartwell and Pember (*10*) in 1918 have shown that the toxic effect of certain acid soils is due not to the true acids but to aluminum salts present in extracts from these acids soils. They checked the effects of aluminum salts added to nutrient solution with acid soil extracts and found the free acids were relatively harmless and that the toxicity was due to the active aluminum. This work was valuable because it made the plant test the final one for the effects of soil acidity.

Mirasol (*18*) has studied aluminum as a factor in soil acidity. He has given a very extensive bibliography of this subject and conducted experiments which showed the beneficial effects of applications of lime and acid phosphate in reducing toxicity due to aluminum compounds. These aluminum salts are claimed to be derived from the acid-soluble aluminum hydroxid, or gibbsite. In soils sufficiently provided with calcium, toxic aluminum salts may never be formed, but in soils deficient in calcium and other bases, such as acid soils, toxic aluminum salts are largely the end products of sulfofication and nitrification. Of

interest also is the fact developed by Conner (*6*) that the quantities of soluble iron in soils are greater when the soils are saturated.

Spurway (*23*) presents data relative to the influence of various salts on the release of soluble aluminum compounds in four soils which he studied. The general tendency of all of the treatments of these soils was to increase the quantities of soluble aluminum, the greatest effect being produced by calcium carbonate and acid phosphate. He reports also that hydrated lime increased the amount of aluminum in the first leaching, but a marked decrease occurred in the second leaching, 15 days after the treatment.

It is evident that the importance of available aluminum salts in the soil is beginning to be appreciated. Aluminum is found in practically all the soils in the Corn Belt, and it is certain, therefore, to become an increasingly important factor in soil management when a system is practiced which does not conserve the calcium and phosphorus salts.

DISTRIBUTION OF IRON AND ALUMINUM IN NORMAL AND DISEASED PLANTS

The tissues in which the metals, iron and aluminum, accumulate are the vascular plate tissues in the nodes of the stalk and shanks and the scutellum of the kernels, as shown in Plate 5. The metals also concentrate in the leaf tissues, as has been determined repeatedly by quantitative analyses. As a rule, the oldest leaves contain the largest quantities of these metals. Gile and Carrero (*9*) have shown that iron is immobile in the oldest rice leaves and in this respect is similar to silicon and magnesium.

The locations of the tissues in the stalk in which iron and aluminum accumulate are those referred to as "zone B" in Plate 3, A. These tissues appear white in normal stalks, with traces of yellow and sometimes green. When the metals concentrate in them they show various gradations of color and of disintegration. Plates 2, B; 5, A and B; and 6 show different kinds of injuries observed in plants in the field.

The extreme toxicity of copper sulphate is seen in Plate 3, B, where the tissues in both zone A and zone B have been seriously affected. This is the only metallic salt studied so far which affects both zone A and zone B tissues. The tissues of the basal nodes, shown in Plate 7, B, are the tissues in which the accumulations occur first. They are the oldest functioning nodal tissues, and their length of service, as indicated by a continued healthy appearance of the roots which have their origin in these tissues, seems to be related to the relative quantities of iron and aluminum which accumulate in them. Part of the basal tissues in the stalk shown in Plate 8, B, are not functioning, as shown by the dry appearance of the lowest and oldest roots. The plant was able, however, to produce additional roots and thereby continue vegetative growth.

In Plate 7, A and B, is shown the base of a stalk which manifested disease symptoms and which was barren. The basal tissues contained abundant accumulations of iron and aluminum compounds. Instead of the metals being distributed more or less uniformly throughout the stalk, as in the normal plant shown in Plate 9, the largest quantities were present in the lower nodal plates and these were brownish purple discolored, and disintegrated, as shown in Plate 7, C.

These two plants illustrate some of the differences commonly noted between plants which are resistant to the development of rots in the roots and stalks and those which become severely rotted. When the plants are vigorous and apparently healthy the quantities of the metals in the nodal tissues are nearly equal throughout the stalk and are not in sufficient quantity or in the state of combination with the plant tissues which causes the brownish purple discolorations.

Furthermore, the accumulation of the metals in the basal nodal tissues of stalks showing symptoms of disease does not necessarily mean a larger quantity of metals in the stalk as a whole. It indicates primarily an unequal distribution of the metals, which can be observed readily by using the microchemical tests, compared with the uniform distribution which is characteristic of normal plants.

The nodal plate from which the ear shoot originates frequently has the largest accumulation of all the nodes in the stalk. When this condition develops early in the life of the stalk the ears may form imperfectly. The nodal tissues may become disorganized and consequently weakened (Pl. 10, B), so that the shanks may break over before the ears are completely matured, as is shown in Plates 6 and 16, B. When this happens the shank scars will show the typical pinkish to brownish purple discolorations, the color varying in intensity according to the time when the discolorations begin to develop in relation to the degree of maturity of the ear.

This condition of the stalk is believed to be a very important factor affecting the rate of maturation of the ears. When the nodal tissues are affected, as shown in Plate 10, B, the ears are delayed in their maturity and tend to remain starchy. It has been found that the distribution of phosphorus also is affected in stalks with these accumulations of the metals. The phosphorus content of the nodal tissues is higher when the iron and aluminum compounds become concentrated in these tissues. The disorganization of the nodal plate tissues interferes with the movement of sufficient quantities of phosphorus and probably other materials to the developing ears and in this way may cause the ears on affected plants to remain starchy. The results of the investigations upon this interpretation of one of the causes of starchiness in corn will be published in a subsequent paper. It is already a well-known fact, however, that adequate phosphates hasten the maturity of the ears.

The mobility of the iron and aluminum compounds seems to be associated with a high sap acidity, according to preliminary tests upon normal and diseased stalks. The sap is distinctly more acid in the normal stalks than in those in which the nodal accumulations of the metallic compounds have taken place. Whether or not the decreased acidity is the result of a greater metal content of the stalks has not been learned.

EFFECTS OF SOIL TREATMENTS UPON THE DESTRUCTIVENESS OF THE ROOTROTS

In Plates 4, 12, A, and 13 the variations in the effects of the available metal salts are seen. The tall, vigorous plants in Plate 12, A, are the more resistant to the rootrots, while the smaller, stunted plants are susceptible and the roots are actually being rotted. It is presumed that these stunted plants have grown from infected seed, yet when infected kernels from the same ears are planted in different types of soil the percentage of damage due to the rootrot is greater in those soils which have the larger amounts of available aluminum.

Marked increases in yield of corn plants have been obtained by various soil treatments. The investigations of Abbott, Conner, and Smalley (*1*), Ruprecht (*19*), Hartwell and Pember (*10*), and Mirasol (*18*) upon different crops show that the addition of lime and phosphorus to the soil and to culture solutions has a beneficial action upon available toxic aluminum salts.

Soil of the same type, as in the field shown in Plate 4, B, was used in the pot culture experiment illustrated in Plate 14, A. Note that limestone was beneficial in producing more vigorous stalks, whereas the addition of aluminum chlorid at the rate of 500 pounds per acre caused the plants to become very susceptible to the rootrots. The limestone treatment of the Hancock County soil, Plate 14, B, was ineffective, whereas acid phosphate produced better plants. The aluminum-chlorid treatment was more harmful than in the Shelby County soil.

In 1918, an experiment conducted at Shelbyville, Ind., showed that the average yield of plants grown from nine healthy ears was increased 35.7 per cent in a soil with a higher lime content, and the average yield of plants grown from five diseased ears grown in ear rows alternating with those from the healthy ears was increased 62.2 per cent.

Results obtained by the Macon County (Illinois) Farm Bureau (*21*) in experiments on the control of corn rootrot by the use of fertilizers and limestone show that when "badly diseased" dent is planted the use of 3 tons of limestone per acre increased the yield of sound corn from 26.07 bushels to 44.43 bushels, or 70 per cent; from 40.13 bushels to 53.98 bushels, or 34 per cent; from 43.37 bushels to 52.90 bushels, or 21 per cent, in plots planted with different strains. The "disease-free" controls yielded at the rate of 49.69 bushels of sound corn in the untreated plot and 60.36 bushels in the treated plot, giving a 21 per cent increase. It should be noted that the "disease-free" corn yielded the highest in both the treated and untreated plots, yet the disease was most destructive in the untreated plots, all of which emphasizes the fact that the highest yields will be obtained when the best seed is planted in soil in the best state of fertility.

Other results reported in various publications have been summarized by Slipher (*20*) and show increases in corn yields due to the applications of lime, but no experiments have been noted which refer to the specific effects on the yield of plants growing from diseased seed.

GENERAL APPEARANCE OF DISEASED PLANTS

The symptoms associated with rootrots in dent have been described by Hoffer and Holbert (*12*), in sweetcorn by Hoffer (*11*), and later and in more detail by Holbert and Hoffer (*13*). These symptoms attributed to rootrots vary considerably because of the wide variations which occur in the type of growth of corn plants. Some strains are hereditarily weaker than others, and, as has already been said, it is believed they possess marked differences in their absorption rates of available salts. No constantly uniform symptoms have been described in open-pollinated corn because of these varying tendencies in the behavior of these heterozygous individuals.

Typical cases of individual plants affected by rootrots are shown in Plates 2, B, and 5, B. Plate 13, A, shows the disease as it occurred in a field in Shelby County in 1918. The stalks had grown to full size, but

shortly after the ears began to develop the roots became severely rotted and many of the stalks fell over. A similar condition of a field of Stowell's Evergreen Sweetcorn is shown in Plate 13, B. Here, too, many of the stalks grew to a good size but after tasseling became badly rootrotted. More of the stalks in this field were of stunted growth than in the Shelby County field, however.

These two cases are characteristic of the occurrence of the rootrots in soils high in organic matter with abundant nitrates but with little calcium and available phosphorus. The nitrates in these soils favor abundant vegetative growth of the plants. The plants under these conditions show discolorations of the nodal tissues very early in their growth, and when they reach the tassel and ear stage of development the nodal tissues frequently are much disintegrated. This disintegration of the nodal tissues results in broken shanks and a weakened root system. The translocation of foods to the roots is retarded and they cease functioning, as shown in Plate 8, B.

The general condition of the plants shown in Plate 4, A, is a very common one in large areas in the Corn Belt. The plants are stunted in their growth, and when they are cut longitudinally and examined internally the nodal discolorations will be found in abundance in the basal portion. The soils in these fields were distinctly acid, relatively low in organic matter, and deficient in calcium and available phosphorus. After the application of 3 tons of limestone to the soil in the field shown in Plate 4, A, the plants grew very well the following season, 1920. The plants in the treated plots were resistant to the rootrots which affected those in the untreated plots.

The plants more resistant to the rootrots grow in soils containing abundant nitrates and adequate calcium and available phosphorus. Calcium carbonate was found to be harmful when applied alone to the soil in the field shown in Plate 15, A. In this field acid phosphate gave the best results. The plants in the calcium-carbonate plot were more severely affected by the nodal accumulations of metals than those in the control plot.

Reference has already been made to the results of the Macon County Farm Bureau experiments (*21*). The rootrot phenomena which develop in plants in that locality are very similar to those shown in Plates 4, B, and 13, B. By the addition of lime and phosphate to this type of soil greater resistance to these rootrots is developed in the corn plants.

It seems from these observations that the quantitative calcium-nitrogen relations which exist in the soils are probably important in connection with the development of the symptoms of rootrots. Whether or not the high nitrogen influences the growth of the plants so that they become susceptible to the rootrots or whether the available metals, which are more abundant under the conditions of high nitrogen and low calcium in the soils, determine the susceptibility of the plants to the rootrots has not been learned. It is probably best to assume for the present that no one of these factors can be fully interpreted independently of the others, so that due consideration must be given to all of them and their interrelations established.

No detailed consideration will be given to the fertilizer experiments conducted as a part of the rootrot investigations in this paper. The results of these experiments will be published elsewhere. It is desired to refer only to the soil conditions in general where the rootrots prevail

and to show that the soil has a marked influence upon the growth of the corn plants and their relative susceptibility to the rootrots.

From the foregoing observations of field conditions under which rootrots prevail, and from the study of the results obtained in experimental plots located in various parts of the Corn Belt, the evidence seems to indicate that the soil environment determines in a large measure whether the plants become severely infected or remain relatively healthy. When kernels from the same seed ears were planted in plots in Iowa, Illinois, Indiana, and Ohio the amounts of rootrot which would develop in the different ear rows was influenced by the soil and growing season more than by the organisms present in or upon the seed planted.

METHODS USED TO DETECT METALS IN STALKS

During the summer of 1919 it was discovered that large quantities of iron compounds, in organic combinations, were constantly present in the nodal tissues which were colored purplish brown. The microchemical method used to detect the iron compounds in these tissues consisted in placing the tissues in a strongly acidulated (HCl) 20 per cent solution of potassium thiocyanate. When the stalks were tested in the field a stellite knife was used to cut them and the solution was applied directly to the exposed stalk tissues. By this means many hundreds of experiments and observations were made upon plants in all stages of development, and it was learned that the brownish purple nodal discoloration was associated with a relatively large quantity of iron compounds which accumulate in these tissues. Invariably the plants which were stunted in their development (Pl. 4, A, B), those whose leaves became streaked with yellow or reddish purple, those with excessive firing of the leaves, those with prematurely broken shanks (Pl. 6, A, B; 16, A, B), and those which fell over early in the field, as shown in Plate 13, A and B, are the plants which constantly are characterized by discolorations and frequent disorganizations of their nodal tissues.

QUANTITY OF ACCUMULATIONS INFLUENCED BY SOIL TYPES

The quantities of iron and aluminum which accumulate in plants grown from kernels from the same seed ear vary according to the nature of the soil in which the plants grow. An experiment was conducted in the station greenhouse in 1919 wherein soils representing types from different counties in Indiana were used, as follows: Soil from the field shown in Plate 4, B, representative of a calcium-deficient soil in Shelby County; soil from Bartholomew County, later found to be lacking in phosphorus; and a soil from Sullivan County, which was deficient in calcium. All pots were kept under relatively uniform conditions of moisture, temperature, and light. A rich garden soil was used as a control.

Table I shows the record of growth of three stalks in each of these four soils. The sign — indicates a trace of iron present in the nodal tissues when tested chemically, × indicates a large quantity, and o indicates no trace whatever. The series of signs represent the nodal content for each node in the stalk with the lowest node in the stalk represented at the left end of the series and the uppermost node in the stalk represented at the right end.

The best plants grew in the garden soil. They were normal green in appearance and had the iron compounds uniformly distributed throughout the stalk. This occurs in stalks of good, vigorous growth. These

results are representative of others obtained by using different kinds of ears, some of which were infected before planting.

These soils, with the exception of the control, were soils in which abundant rootrots prevailed during the season of 1919 under field conditions in these representative counties. The stalks were cut longitudinally and then tested for iron compounds. At the same time the length of the stem from the growing point to the base was measured. This serves as the best index for showing relative amounts of stalk growth. The soil influence upon the distribution of iron is very marked in the different stalks, as is seen in Table I.

TABLE I.—*Distribution of iron in vascular plate tissues in the nodes of three stalks grown from seed from the same seed ear in each of four different soils, under otherwise similar conditions for growth, at La Fayette, Ind., 1919*

Source of soil.	Height of plant.	Distance from growing point to base.	Color of leaves.	Relative distribution of iron in vascular plate tissues.[1]
	Cm.	*Cm.*		
Garden	150	30	Normal green	— — — — — — — —
	150	40	do	— — — — — — — —
	120	25	do	— — — — — — — — —
Shelby County	80	12	Slight yellowish green.	× × × — — — — —
	100	16	Green	× × × × × × — — —
	90	16	Green	× × × × × × × × ×
Bartholomew County	75	15	Yellowish green	× — — — — — o o o
	100	20	do	× × — — — — o o o o
	90	22	do	× × × × — — o o o o
Sullivan County	95	20	do	× × × × × × × — — —
	80	6	Normal green	× × × × — — — o
	80	4	do	× × × — o o o

[1] ×=relatively large quantity of accumulated metals in nodes.
—=trace.
o =no trace.

There were cases, however, where the plants in some soils were apparently diseased and would not show the brownish purple discolorations of the nodal vascular plate. When tested for iron compounds no considerable quantities would be found in the nodal tissues. During 1920 the microchemical method of detecting aluminum by use of logwood[3] was applied, and it was then discovered that aluminum was present in these tissues in large quantities. After the usual test for iron, the aluminum test was made, and the results from the two tests usually suggested that aluminum may be equally as important or more so than the iron in its action upon the tissues. This test cleared up what appeared to be discrepancies in interpretation of the results in the field when nodal tissues would be found in stages of disintegration but without the usual amount of iron present in the tissues. The aluminum test would indicate the total quantities of both metals present in the tissues.

INJECTION EXPERIMENTS

Experiments were planned and carried out during the summer of 1920 to determine the rôle of aluminum in the cornstalk, and whether or

[3] The logwood test consists in boiling the tissue to be tested for aluminum in a saturated solution of ammonium carbonate which has been colored deep red by logwood. This test should always be made after the relative quantity and distribution of iron in the tissue has been determined by the thiocyanate test, using a single piece of tissue for each test.

not its action was comparable to that of iron. Injections of various salts into normal-appearing stalks were made and the specific effects of single salts and of salts in various combinations were observed. In all cases a hill with two similar and apparently normal stalks was selected. The stalk not treated served as a control for comparison with the treated stalk.

METHOD OF INJECTING THE SOLUTIONS

The method which was used to introduce the chemicals into the plants for the study of their specific effects on the plant tissues was to insert a straight calcium-chlorid tube into the stalk just above the first node. The stalk was first punctured with a sharp cork borer of the same size as the small end of the tube. The tubes were inserted approximately to the center of the stalk and then slightly exserted to form a small reservoir for the solutions so that they could be absorbed by some of the severed vascular bundles. Measured quantities of the test solutions were then placed in the tubes, and a stopper was placed loosely into the distal end. The method is illustrated in Plate 12, B.

When the cortical tissues of the stalk were imperfectly cut the solutions would leak out, and in such cases the results were ignored. Only those cases where definite absorption of the solutions occurred have been considered. The quantities of the solutions which remained in the tubes at the end of the experiment were measured, and the total amounts were recorded. The quantities which were absorbed varied considerably for different solutes and the moisture content of the soil.

Because of the wide range in the sizes of the plants which were treated and because of their heterozygous conditions also, no accurate quantitative studies have been considered worth while yet with the strains of corn available for study. Only plants which were apparently normal were used, and each group of plants experimented upon had their origin from the same seed ear. This tended to reduce the strain differences to a minimum.

The injections were begun just at the time the plants began to tassel. They are difficult to make prior to this time because the cortical tissues are not usually mature enough to support the tubes rigidly. The relative proportion of the amount of stalk material to the quantity of solute injected can not be accurately determined because the specific effects on the tissues of varying quantities of the solute are dependent upon a definite time relation. This, too, is affected by changing plant-volume and absorption-rate entities during the time when injections are made.

The metals which accumulate in the plants under normal seasonal conditions are absorbed by the plants in very dilute concentrations from the soil solution. The concentration of the metallic salts in the soil solution is therefore a most important factor in determining the length of time required to produce the specific effects of the metals. This difference in availability of these salts is often observed in fields within small areas. In Plate 17 the effects are shown on plants of dent and sweetcorn, respectively, grown from seed taken from the same seed ears. The plants grew within 12 feet of each other, the stunted plants being taken from the ends of the rows in the soil with larger quantities of available metals. Sweetcorn is markedly more affected by these soil conditions than dent. This has been observed many times in districts where both kinds are extensively grown.

For the reasons discussed, relatively high concentrations of some of the solutions were used. It should be indicated that the quantities of injected solutions absorbed are relatively small in comparison with the quantities of the soil solution and other solutes which are absorbed by the plants. Neither is the quantitative complex of nutrients in the cell sap known. The injected solutions represent additions to the actual quantities absorbed by the plants. Obviously, it is difficult to study the effects of the various solutes in the stalks in the field. Therefore, the chief value of the injection experiments was to learn what effect the various ions had upon the plant tissues when injected into them in addition to those which were already present in the sap.

The results of the injections are given in Table II. The solutions were mostly 1 per cent concentrations of the various solutes. The characteristic effects of the iron and aluminum salts are especially noteworthy. The effects are referred to in Table II as being either injurious or causing no injury but their specific actions will be described in greater detail in the text.

TABLE II.—*Effects of solutes injected into corn plants, based on the average results from five similarly treated stalks, all of the same age and approximate size, from readings made seven days after injection*

Solute.	Strength.	Average quantity injected.	Result.
	Per cent.	*Cc.*	
Aluminum chlorid.	1	5	Completely fired and streaked; nodes destroyed.
		19	
Aluminum nitrate.	1	5	Leaves streaked, partially fired; node destroyed.
		10	Do.
		15	Leaves streaked; fired; nodes destroyed.
Calcium nitrate....	1	20	No apparent injury.
Aluminum nitrate.	1	10	No apparent injury to leaves; nodes slightly discolored.
Calcium nitrate....	1	15 (25)	
Aluminum nitrate.	1	12½	Leaves streaked; necrosis of intervascular tissues; nodes slightly disintegrated.
Calcium nitrate....	1	12½ (25)	
Aluminum nitrate.	1	15	Streaked; necrosis of intervascular leaf tissues; and disintegration of nodal plate tissues.
Calcium nitrate....	1	10 (25)	
Sulphuric acid.....	N/5	30	Plant killed; nodal tissues destroyed.
	N/10	30	Plant apparently normal.
	N/20	30	No apparent injury.
Hydrochloric acid.	N/20	30	Do.
Phosphoric acid....	1	200	Do.
Tartaric acid.....	1	10	Do.
Citric acid.....	1	20	Do.
Acetic acid.....	1	30	Do.
Malic acid.....			
Formic acid.....	1	30	Plant killed; nodal plate tissues destroyed.
Iron citrate.....	0.25	30	Very slight marginal firing.
	0.5	30	Plant killed; nodal tissues brown.
	1	30	Do.
	5	30	Do.
Ferrous sulphate...	1	30	Leaves purple-streaked, the streaked tissues firing with a distinct gray color.
Iron citrate.....	1	5	No apparent injury.
Magnesium acetate.	1	20 (25)	
Iron citrate.....	1	10	Leaves purple streaked; nodes discolored.
Magnesium acetate.	1	15 (25)	
Iron citrate.....	1	5	No injury.
Calcium citrate....	1	20 (25)	
Iron citrate.....	1	12½	Purple streaks on leaves; nodes discolored.
Calcium acetate....	1	12½ (25)	

Aluminum-chlorid, aluminum-nitrate (Pl. 11), iron-citrate (Pl. 18, A), and ferrous-sulphate injections (Pl. 18, B) caused marked injurious effects upon the vascular plate tissues, while iron nitrate (Pl. 18, C) and iron chlorid (Pl. 18, D) accumulated in the vascular plate tissues without producing any marked injury to either the leaves or vascular plates. These accumulations were detected by testing the tissues chemically.

Acetic, citric, tartaric, phosphoric, and malic acids in 1 per cent concentrations caused no injurious effects that could be noticed in the plants. In fact, the plants receiving the malic and phosphoric acids appeared greener and healthier than the control plants. Formic acid, however, proved to be harmful, the plants dying within two days. The rôle of the organic acids in relation to the availability of the metallic salts is being investigated further. Mazé (*16*) reports that malic acid is secreted by the roots of the corn plants and has an important bearing upon the absorption of the plant nutrients.

The chief purpose of the injection studies was to determine the specific effects of the iron and aluminum salts. The effect of aluminum nitrate upon the corn plant tissues is shown in Plate 11, A and B. The ultimate effect upon the leaf tissues consists of a killing of the intervascular leaf tissues and a destruction of the nodal vascular plate tissues. The progress of this action on these tissues is of much interest because the symptoms of various stages resemble closely certain of the symptoms which become prominent during the progress of development of the injuries in the stalk and leaves associated with the rootrot disease in soils with available aluminum salts.

Of a 1 per cent solution of aluminum nitrate 5 c. c. was sufficient to cause the effects shown in Plate 11, A. The quantity of aluminum ions was less than 9 mgm. It is seen then that a sudden increase in the aluminum-ion content of the stalk is disastrous and that the quantity that is necessary is exceedingly small.

Aluminum chlorid is also very toxic, but in this case the action of chlorine ions may have played a part in the injuries. These effects will be studied further. In this relation the effects of *N/20* hydrochloric acid should be noted. No injury to the nodal plate tissues resulted from the introduction of 30 c. c. of the acid. From these results it is evident that the aluminum ions are the toxic ones.

When *N/100* aluminum nitrate was injected into the stalks the injury to the leaf tissues developed slowly, the first effect being a slight yellowing of the intervascular tissues at the distal end of the leaves. The yellowing of the tissues was followed by a complete disappearance of the chlorophyll and this by a water-soaked appearance of the tissues. The affected tissues then became dry and brown, and the leaves presented the streaked appearance shown in Plate 11, B. The stages in the progress of the injury to the nodal tissues were observed in a number of plants. The accumulation was most pronounced in the vascular plate tissues, although the aluminum salts combine with the cell contents of the phloem cells along the entire vascular bundles through which they are being conducted. The vascular plate tissues (zone B in Pl. 3, A) first become yellowish brown in color, then brown, and with the deepening of the brown color the disintegration of the tissues can be noted. The advanced stages of this disintegration are shown in Plate 11, A.

SOIL CONDITIONS WHICH FAVOR ALUMINUM AVAILABILITY

The conditions which develop in a soil to make the aluminum salts available also account for increased quantities of available iron salts, and, therefore, salts of both elements are absorbed at the same time. Because of this close chemical relation of these two metals it is necessary to study much further the effects of fertilizers and soil acidity upon their respective availabilities to the corn plants. The fact that chemical tests of diseased and normal stalks in the field show variations in the accumulations of compounds of each metal in the stalks in the nodal tissues suggests that the specific effects of the metals upon the discoloration and rate of disintegration of the vascular plate tissues may be determined by certain conditions in the soil or by variations in the "elective" capacities of different plants to absorb quantities of these metallic salts when available.

It is important, therefore, that the corn plants in the seedling and juvenile stages of growth should be least affected by the available metals. Acid phosphate applied to the soil at planting time greatly stimulates the growth of young plants in experimental plots. Whether this is due to the increased phosphorus or to the precipitation of the available aluminum which might affect the plants is uncertain, yet it is known that aluminum toxicity takes place in the absence of sufficient phosphates to precipitate the aluminum salts in the soil solution. And, furthermore, the plants in the youngest stage of development would be most rapidly affected and therefore show the effects of toxicity in retarded growth.

TISSUES IN WHICH THE METALS ACCUMULATE

By treating the vascular plate, zone B tissues, in healthy plants with concentrated nitric acid, the cells where the accumulations of the organic metallic compounds occur first will give a distinct xanthoproteic reaction. The cells lie close to the xylem elements of the branching bundles in the vascular plate. When the disintegration of the tissues begins, these cells are the first ones to break down.

This disintegration effect on the zone B tissues may have much significance although it must be admitted that very little is known regarding it at the present time. When the metals accumulate in these nodal tissues after their injection into the stalks it has been found that the nodal tissues in zone B, as shown in plate 3, A, give a strong test for peroxidase with gum guaiacum. This reaction is probably of much significance and may indicate in a measure the probable cause of the discolorations which are consequent to the increased metallic content of these tissues.

Bayliss (*3*) reports that—

> a peroxidase is in all probability a peculiarly active form of the colloidal hydroxides of manganese, iron, or copper preserved in this active state by the presence of an emulsoid colloid, such as gum or albumin.

He states further that the metals found to be active as peroxidases are those capable of existing in two states of different valencies. If this conclusion of Bayliss is correct it would assist in interpreting the phenomena observed in the nodal vascular plate tissues in the treated stalks and in diseased stalks. The fact that the iron and aluminum accumulations are organic aggregates of some kind in these tissues, and the fact that the tissues become strongly active enzymatically, with special reference to peroxidase, may be significant. The intensity of this action probably would determine the period of functioning of these tissues. The

increased peroxidase activity in the tissues may yield hydrogen peroxid, or other by-products, which in themselves may become toxic and account for the destruction of the tissues consequent to the metal accumulation. Furthermore, other metals and organic substances may enter the plant and stimulate this peroxidase activity in the zone B tissues. After the basal tissues and roots begin to rot the decomposition products from these rotted parts may be absorbed and act as toxins in the plant. These suggestions are given because the resultant effects of the root and stalk rots upon the plant present a complexity of phenomena which are difficult to analyze.

On the other hand, the fact that the metals accumulate in the cells giving a strong xanthoproteic reaction may confirm the report of Szücs (*24*) that aluminum combines directly with protoplasm and "sets it." Loew (*15*) infers that calcium-protein compounds exist in the organized particles from which the nucleus and chlorophyll bodies are built up. The replacement of the calcium by another element would lead to a disturbance in the structure of the protein molecule, and this would prove fatal.

Whether or not calcium-protein aggregates exist in the cells in zone B tissues as well as in the leaf tissues, it is of much significance that when calcium was introduced along with the aluminum salts the injuries to the nodal and leaf tissues were reduced in severity during the length of time necessary for the aluminum ions alone and in equivalent concentrations to become actively toxic. The mass effect of a preponderance of calcium in the cell tissues may inhibit the disorganization of the protein aggregates by the aluminum and iron salts.

The iron-citrate solution was absorbed readily by the stalks and killed the plants within three days. The characteristic effect of the iron citrate was a rapid wilting of the stalk and a heavy precipitation of iron compounds all along the vascular bundles, as shown in Plate 18, A. The leaves developed a vinaceous gray color, and the tissues next to the vascular bundles in the stalk and leaves became brown.

Ferrous sulphate was probably the most actively toxic form of iron salts tested (Pl. 18, B). The dark brown discolorations along the vascular bundles in the leaves are characteristic of the first effects of ferrous sulphate, and this is followed by a wilting and the death of the leaves. The vascular bundles in the stalk become brown, and in zone B disintegration of the tissues occurs. The color of the darkened tissues is a brownish purple, not unlike the discolorations found in stalks growing in acid soils, as shown in Plates 2, B, and 5, A and B. The disintegration of the tissues, of course, is much more rapid than that which occurs naturally in the field, due to the stronger concentrations of the solution used. The most abundant nodal discolorations do not appear in the stalks until tassel time, a period of probably 60 to 80 days after the seed is planted, although seedlings growing in soils with large quantities of available aluminum and iron will show the basal tissue discolorations very early, according to their different relative absorption rates.

Because of the gradual and progressive changes which can be induced in the corn plants by the injection of dilute solutions of aluminum and iron salts and because these so closely resemble the progress of development of similar symptoms in corn plants growing in different types of soil, it is evident that the available salts of aluminum and iron in most soils are absorbed by the plants in dilute subtoxic concentrations and enter into organic combinations with various tissues in the plants. With

continued absorption of these metals the cumulative capacities of the plants seem to reach a limit, and then the organic metal aggregates in the cells begin to disintegrate. The rate of this action may be determined by the quantities of alkaline bases present in the plant sap. The fact also that the metals become "masked" or fixed in the plant tissues would probably explain their continued absorptions from the soil solution, the rate of absorption being largely determined by their relative availabilities.

QUANTITIES OF ALUMINUM PRESENT IN STALKS FROM DIFFERENT LOCALITIES

The percentage of ash in cornstalks varies considerably for plants grown in different parts of the country. Analysis of a limited number of plants shows that their ash contents vary from approximately 3 to 6.3 per cent. The aluminum content of the ash of the leaves also varies markedly, within a range extending from 1.75 per cent to as high as 5.06 per cent in some plants. In fact, the aluminum content of the ash of diseased corn plants obtained in 1920 from the Wanatah (Ind.) soil investigated by Abbott, Conner, and Smalley (*1*) was over 10 per cent.

Table III contains a summary of the analyses of the ash and aluminum contents of normal and rootrotted plants selected in different localities. The aluminum content has been constantly greater in the diseased stalks than in the normally growing ones. These differences suggest a relation between the occurrence of the rootrots and the aluminum content of the stalks.

TABLE III.—*Percentage of ash and aluminum in normal and diseased stalks obtained from different localities in 1920*

Locality.	Normal.			Diseased.		
	Number of stalks.	Percentage of ash.	Percentage of Al_2O_3.	Number of stalks.	Percentage of ash.	Percentage of Al_2O_3.
La Fayette, Ind.	3	3.22	1.77	11	3.51	2.80
Sullivan, Ind.	3	2.99	2.00	8	4.48	3.32
Amherst, Mass.[a]	.....	.....	.....	8	6.27	4.27
Battleground, Ind.	2	4.71	2.21	3	4.27	5.06

[a] Diseased specimens only were received.

TABLE IV.—*Total ash content and percentage of aluminum in healthy and diseased stalks of Reid Yellow Dent from experimental plot at Battleground, Ind., in 1920*

	Ash.	Al_2O_3.
	Per cent.	*Per cent.*
8 healthy stalks	3.64	1.99
21 diseased stalks	4.09	3.73

Table IV gives a direct comparison between specially selected stalks of Reid Yellow Dent variety. Various types of stalks showing symptoms of rootrots were selected and checked against the normal-appearing stalks growing in the same soil with the same conditions for growth.

It has been learned that certain soils vary within a few feet, and for this reason some of the plants which became diseased may have had larger quantities of these salts for absorption. This soil condition is frequently found in many fields, as is illustrated in Plate 4, A.

Plate 17 shows two plants growing from the same seed ear. The plants were grown within 12 feet of each other. The soil in which the normal plant grew gave no reaction for available iron and aluminum, while the soil in which the stunted plant grew had a lime requirement of 1,100 pounds per acre. Plate 19 shows this marked difference in the rates of growth in a field. The tall plants were growing in a soil which gave no reaction for available iron or aluminum and also reacted basically to the extent of an equivalent of 600 pounds of lime per acre. A sample taken from the soil where the stunted plants were growing showed the presence of available aluminum and iron salts, and a lime requirement of 900 pounds per acre. The plants in this part of the field were badly rootrotted on June 30, 1921, while those growing in the better soil had no rot lesions on the roots.

The ash of normal stalks contains approximately 2 per cent of aluminum ($Al_2O)_3$, but when the proportion increases the pathological phenomena of stunted growth, leaf streaking, nodal-tissue discoloration and disintegration, broken shanks, and premature death of the stalks may develop. The nature of the symptoms, of course, is dependent upon the relative availability of the aluminum and iron salts in the soil and the meteorological conditions favoring the growth of the corn plants.

EFFECTS OF METALS ON FUNCTIONING OF VASCULAR BUNDLES

Many plants which wilted and died prematurely have been studied. When stalks in various stages of disease are cut near the base and placed immediately in tap water colored with methylene blue it will be found that a number of the vascular bundles are not functioning. Plate 20, A–C, and Plate 1, B, show longitudinal and cross sections through a diseased stalk. Note the small number of functioning bundles as contrasted with sections through a similarly treated normal-appearing stalk, as shown in Plate 21, A and B, and in Plate 1, A. It has been found that the central bundles in the stalk cease functioning first, and when longitudinal sections are made through the stalks and tested chemically for iron and aluminum, these are the ones which give the reactions for the largest quantities of accumulated metals in the vascular plate tissues.

This plugging of the vascular bundles is a common effect of various solutions of the metals when absorbed by corn plants. Aluminum salts are especially active in this regard. It has been found in experiments that plants wilt very rapidly when cut and placed in *N/100* solution of aluminum nitrate, whereas in the same concentration of ferrous sulphate larger quantities are absorbed before wilting begins. This latter type of wilting, due to ferrous sulphate, is subsequent to marked leaf-tissue injuries, whereas the wilting due to the aluminum usually precedes the leaf-tissue injuries. The physiological-chemical interpretation of this action is not known. Neither is the physiological effect of the metal salts upon the phloem tissues known. The changes which occur in plants with large quantities of metals in their tissues offer many problems which must be solved according to their influence upon the functions of the different tissues, such as absorption, transpiration, translocation,

and general metabolism. It is known that they act in a beneficial manner upon certain tissues when present in very small quantities. When the quantities are supra-optimal the effects become pathological.

The influence of the metals which frequently accumulate in the scutellum of the kernels, as shown in Plate 5, C, upon germination and growth of the seedlings must also be considered. There is some evidence to indicate that they affect the amylase activity in these tissues and inhibit the growth of the young seedlings.

Many weakly germinating seedlings have been studied in this regard, and it has been found that many seedlings which possess a darkened scutellum, such as has been described by Adams and Russell (*2*), will show relatively large quantities of accumulated iron in these tissues in comparison with the quantities which normally are present in the seedlings of high-yielding, disease-free types. Whether or not these tissues are affected by the metals so that they too become more easily invaded by fungi has not been determined, yet they frequently are infected by *Rhizopus nigricans* Ehrb., *Penicillium* spp., and other organisms.

The conditions of high aluminum availability in acid soils are chiefly those which occur when calcium and phosphorus salts are lacking, as shown by Mirasol (*18*). For this reason the effects of aluminum compounds may rapidly become injurious, due perhaps both to a cumulative toxicity in the concentration centers in the plants and to a phosphorus starvation of the growing and functioning tissues.

The influence of nitrates in the soil has already been referred to. When they are abundant the plants seem to grow more vigorously and the quantity of aluminum compounds may not reach toxic proportions in any particular part until the plants have made good vegetative growth. When the available nitrates, as well as the calcium and phosphorus in the soil, are low, as shown in Plate 4, A and B, and Plate 19, the cumulative aluminum toxicity develops rapidly and the growth of the plants is retarded. The nodal tissues become disorganized very early in the life of the plant. Plants in the condition shown in Plate 19 have recovered from this stunting effect after heavy rains. New roots may push out, provided the basal tissues are not too badly affected, and with a decreased percentage of available salts of aluminum and iron in the soil more rapid growth may take place. Any retardation of the early growth of the stalks due to any cause, either the accumulated metals, infected seed, or insect injury, results in a lessened productive capacity of the plant.

IMPORTANCE OF AVAILABLE PHOSPHATES

As the quantities of available aluminum compounds vary according to the type of the soil and according to the farm management practiced upon the different soils, and, furthermore, as the amount of moisture affects the quantities of available aluminum compounds, as has been shown by Conner (*6*), it is evident that the rate of absorption of these salts must be related to their respective availabilities during different periods of the growing season. The other available elements, calcium and phosphorus, also vary in quantity in the different soils. The phosphorus relation is the most important one in connection with the aluminum and iron salt availabilities. The aluminum salts are not available in any considerable quantities in a soil containing soluble phosphates. It is a recognized fact that most acid soils are deficient in phosphates.

The greater the intensity of the acidity the greater are the quantities of aluminum and iron salts which become available.

When lime is added to acid soils it is believed that the conditions which operate to make the aluminum and iron compounds available are destroyed, and even though aluminum salts may still be available after calcium carbonate is added, the addition of soluble phosphates will rapidly precipitate the aluminum salts and render the aluminum inert so far as absorption by corn plants is concerned.

Lime has a beneficial effect according to Hartwell and Pember (*10*), Mirasol (*18*), and Conner (7), although its action is markedly slower than that of acid phosphate.

Aluminum phosphate is relatively insoluble, yet when used as a fertilizer it produces an increased growth of corn plants, according to unpublished results obtained by Conner. This is perhaps due to the hydrolysis of the aluminum phosphate, as shown by Cameron and Bell (*4*), the result of which would yield inert aluminum hydroxid and available phosphorus to the plant.

These statements are based upon studies of plants in fertilizer experiments in the field and greenhouse.

DISCUSSION

The rôle of phosphorus in plant growth and reproduction is a problem which has been worked upon by many investigators, and its relation to certain of the metabolic processes is fairly well understood. The ash content of kernels of corn averages 45 per cent phosphoric acid. Its importance, therefore, in the production of seed corn needs no further emphasis.

The fact that phosphorus is deficient in most acid soils is also well known and needs no discussion. The importance of available aluminum compounds in the soil, however, is just beginning to be studied intensively, and the fact that when the aluminum salts are available or become available the supply of available phosphates is not adequate for the needs of plants in normal growth is one which presents a complex problem for investigation.

The rôle of lime in the soil in relation to the conditions under which aluminum and iron salts become available for absorption is probably that of a stabilizer of the soil reaction. The hydrogen-ion index of a black loam soil deficient in phosphates and lime varied markedly during the growing season of 1920. Therefore, if the soil reaction can be maintained constantly near neutrality the quantities of available aluminum and iron salts will be less for absorption by the corn plants.

The growth phenomena of the corn plants can not be normal under such conditions of deficient phosphates and lime and available aluminum and iron salts. It is necessary, therefore, to isolate the phenomena due to specific ions, if possible, and study them separately. This was attempted in part by injecting solutions of supposedly harmful salts and others into normal plants. By this means it was found that aluminum and iron ions were harmful and would produce effects similar to those which become manifest in many stalks which are affected by rootrots. A specific toxic action was stimulated by the aluminum and iron ions, respectively. Organic and mineral acids injected into the stalks in higher concentrations than are ever found in acid soils produced no specific results. Furthermore, when calcium ions were introduced in

large quantities the intensity of the action of the metal ions seemed to be inhibited during the time usually required in the experiments for the aluminum and iron ions to become injurious.

These injection experiments have their shortcomings. The salts absorbed naturally by the plants, as well as the quantity of water, are unknown quantities. For this reason the concentration of the specific ions after injection into the plant could not be approximated. The resultant effects of introducing definite quantities of solutes irrespective of their dilution were studied. By this means the cumulative toxicity of specific aluminum and iron ions was definitely proved, although the toxic and lethal concentration within the stalks are unknown. Probably the aluminum and iron ratio to the green weight of the plants offers the best basis for comparison, but this must be definitely established. With a large quantity of available metals during the seedling stage the ratio would be very high, but with rapid growth of the plants under favorable meteorological conditions and abundant nitrates the ratio may be kept relatively low until later in the life of the plant. Furthermore, different plants will absorb the metals at different rates, and this absorption-rate capacity of the plants also complicates the problem.

The brownish-purple discolorations and frequent disintegrations of the nodal tissues are the result of the metal accumulations in these tissues. Because of the soil relations which have just been described, it seems reasonable to conclude that when the accumulations of metal, especially aluminum, occur in abundance in the corn plants, the soil is deficient in available phosphates. Furthermore, under these conditions if the organisms commonly found in rotted roots, such as *Fusarium moniliforme*, *Trichoderma* sp., *Rhizopus* spp., *Penicillium* spp., a white bacterium, and others, are present in the soil they will produce definite rot lesions in the roots and basal parts of the stalks. The condition of the tissues within the plants seems to determine the rate of development and consequent injuriousness of these rots.

Resistance to the rootrots in corn plants seems to be defined (1) by the respective abilities (inherited) of the plants to absorb materials present in the soil solution and (2) by the relative concentrations of deleterious ions presented to the roots in the soil solution.

In this consideration of the rootrots of corn it should be borne in mind that the importance of using seed which has not been damaged by infection by fungi and bacteria is in no way diminished. The value of using seed which has not been weakened by infections has already been indicated (*11*, *12*, *13*). These investigations cover those effects upon plants which occur irrespective of the seed infections and which are more directly referable to the soil influences upon the growth of the plants and their relative resistances to these influences which determine susceptibility to and the extent of development of the rots in the plant parts. If these infections can be controlled in a large measure by soil treatments and by breeding better strains of corn, the practical importance of these investigations will be established. Therefore, seed ears should be selected which are free from infections and brownish purple discolorations of the shank tissues. Moreover, the plants in the field which do not show accumulations of large quantities of the metals, as evidenced by the nodal plate discolorations and disintegrations of the tissues, by the time the ears are matured, are likely to be of greater value to the corn breeder than those plants which show these characteristics when grown under similar conditions. These statements apply

equally well to plants in all localities, irrespective of whether rootrots develop in them and appear to become a serious menace to the crop.

SUMMARY

(1) One of the most characteristic differences between normally growing corn plants and those which become severely rootrotted is the condition of the vascular plate tissues in the nodes of the stalks. The plants which become severely rootrotted are those which have the nodal tissues discolored and in various stages of disintegration.

(2) This disintegration of the nodal plate tissues begins in the absence of any specific organisms in the tissues.

(3) The brown, yellowish brown, and brownish purple discolorations with their consequent disintegrations which are frequently found in diseased plants have been produced artificially by injecting solutions of certain salts of aluminum and iron into the plants. Definite chlorophyll and leaf-tissue changes have been produced also. Other factors, however, may operate to produce similar effects.

(4) These artificially induced changes in the plant parts closely resemble the phenomena which develop in plants growing in the field under conditions favorable to rootrots.

(5) The most severe cases of rootrots have been found in soils notable because of their deficiencies of lime and available phosphates.

(6) Such soils have variable quantities of salts of aluminum and iron available for absorption by plants.

(7) Corn plants show marked differences in the quantities of aluminum and iron salts which are absorbed by them. These differences develop when the salts are available in subtoxic concentrations in the soil and are believed to be due to specific selective capacities of different plants to absorb the available aluminum and iron salts from the soil. This type of selective absorption cannot operate when the aluminum and iron salts occur in quantities which are toxic to the roots.

(8) A definite cumulative toxicity of aluminum salts within the plants was established by the injection experiments, and it is believed that the same phenomenon occurs naturally in the field. The relative quantities of the available metals and of nitrates in the soil determine, in a large measure, the rate of development of the cumulative toxicity of the metals within the plants. Those plants which contain the largest quantities of these metals are the ones which seem to develop the most severe cases of rootrots when the organisms are present in the soil and the meteorological conditions favor their optimum growth.

(9) When abundant aluminum injuries occur in the corn plants in certain fields it is an indication that the soil is deficient in available phosphates.

(10) The application of lime and phosphates to soils in which rootrots have developed in destructive proportions has been decidedly beneficial in controlling them. The use of limestone alone in some instances proved harmful, but in all cases studied so far the application of available phosphates produced plants which were better and more resistant to the rootrots.

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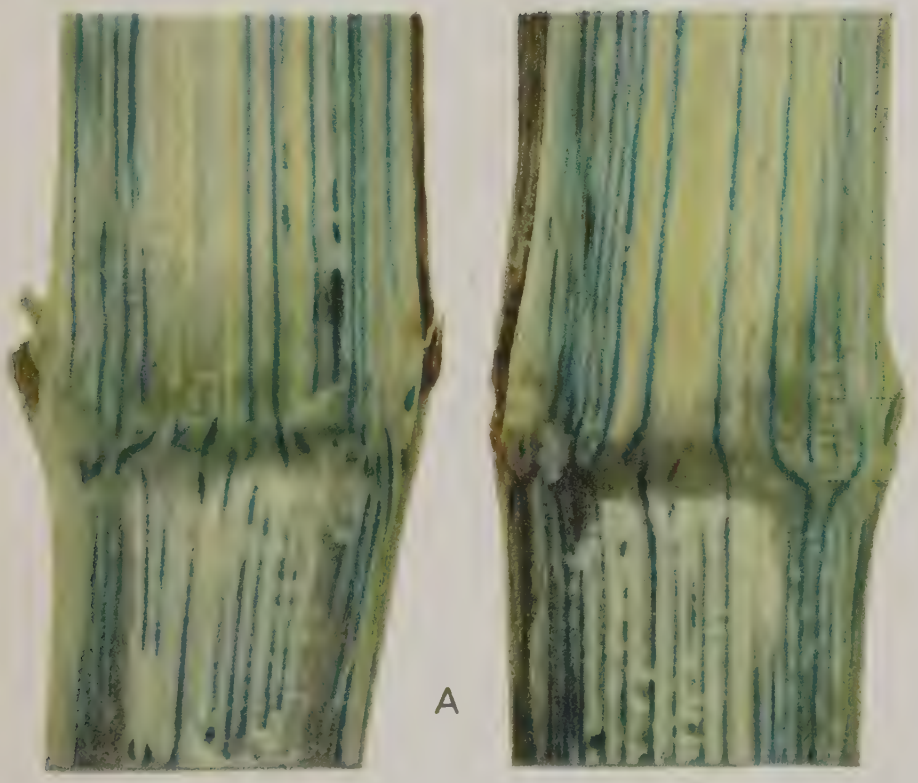
PLATE 1

A.—Longitudinal section through a node of a normal stalk. Most of the bundles were functioning, as is indicated by their staining with methylene blue. Very few bundles tested for iron and aluminum in zone B.

B.—Longitudinal section through a node of a diseased stalk. Very few of the bundles were functioning. The inactive bundles contained large accumulations of iron (red) and aluminum in the zone B region. This is characteristic of stalks showing symptoms of rootrots.


Zone A
Zone B
A

Zone A
Zone B
B

A

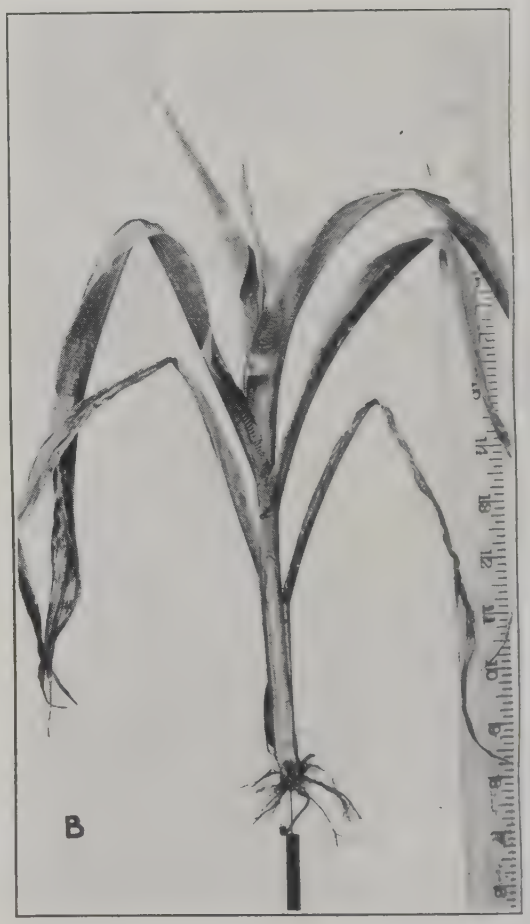

B

PLATE 2

A.—Base of a normal stalk of yellow dent grown in a well-fertilized soil. The basal tissues are free from discolorations and fungi. The soil showed no available aluminum salts when tested.

B.—Stalk of yellow dent in which there are large accumulations of aluminum and iron compounds. The roots are rotted and the basal tissues invaded by fungi and bacteria. This plant was taken from a soil notably deficient in available phosphate but containing available iron and aluminum salts.

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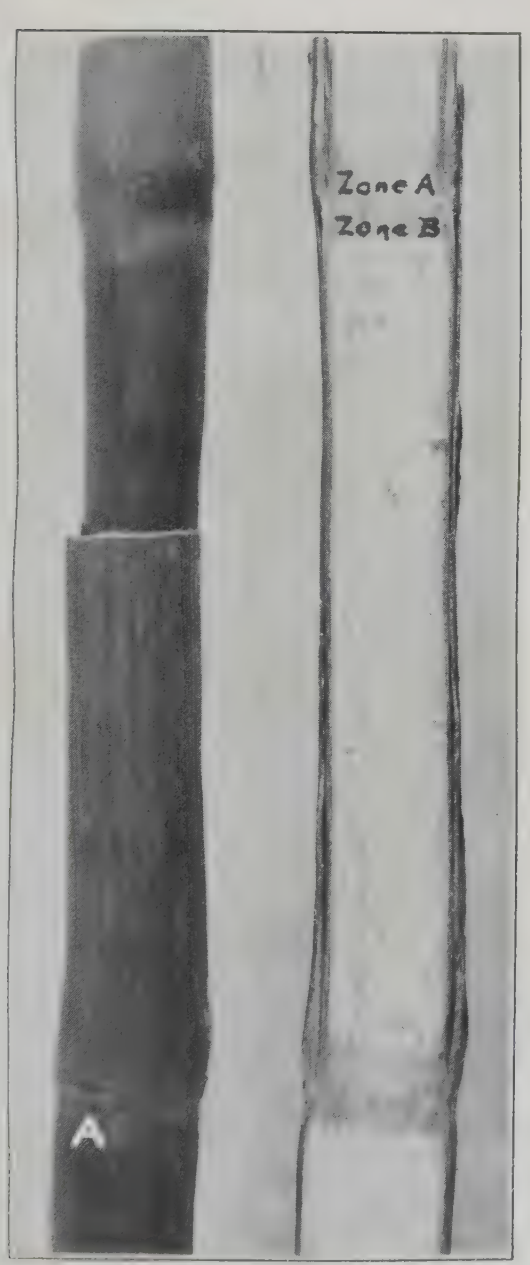
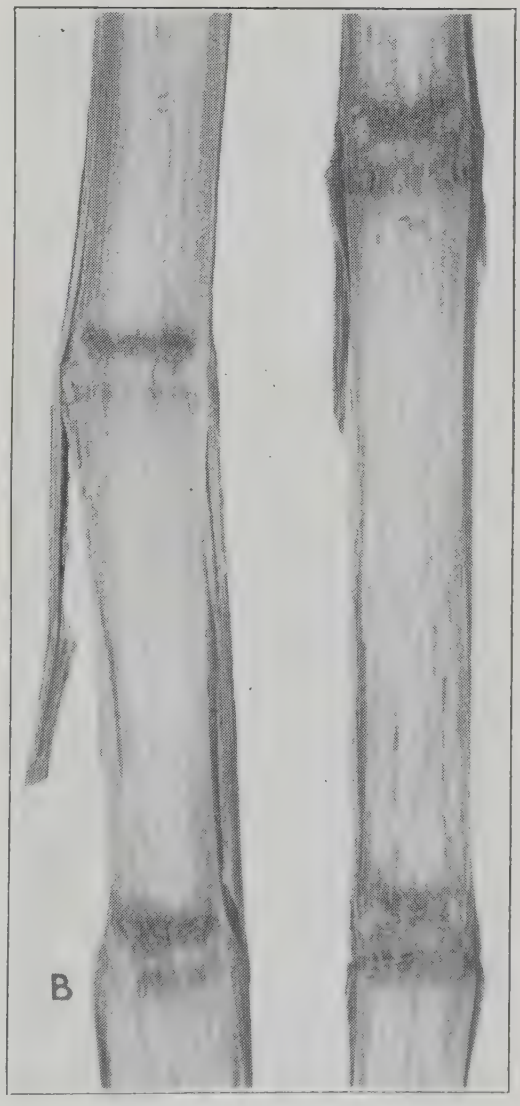
PLATE 3

A.—The nodal tissues can be classified into two zones, zone A, the growing region, and zone B, the vascular plate region. Iron and aluminum compounds concentrate in certain tissues in zone B.

B.—Copper sulphate in $N/100$ concentration caused injuries to the tissues in both zone A and zone B when injected into the plants. Copper, aluminum, and iron salts produce similar effects upon these tissues in different intensities.


Zone A
Zone B
A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A

B

PLATE 4

A.—A field of Johnson County White Dent in Sullivan County, Ind., in 1919. The soil was acid and had a high available aluminum and iron content. Three tons of ground limestone applied in 1920 corrected this condition, and the plants in the treated areas grew better and were more resistant to the rootrots.

B.—A field of Country Gentleman Sweetcorn in Shelby County, Ind., in 1919. The plants in the foreground showed abundant rootrot at this stage of development. In the background in a heavier soil the plants were in tassel and were markedly less affected. The soil when tested showed large quantities of available aluminum and iron salts. It was deficient in lime and available phosphates. This type of soil was used in the pot cultures shown in Plate 14, A.

PLATE 5

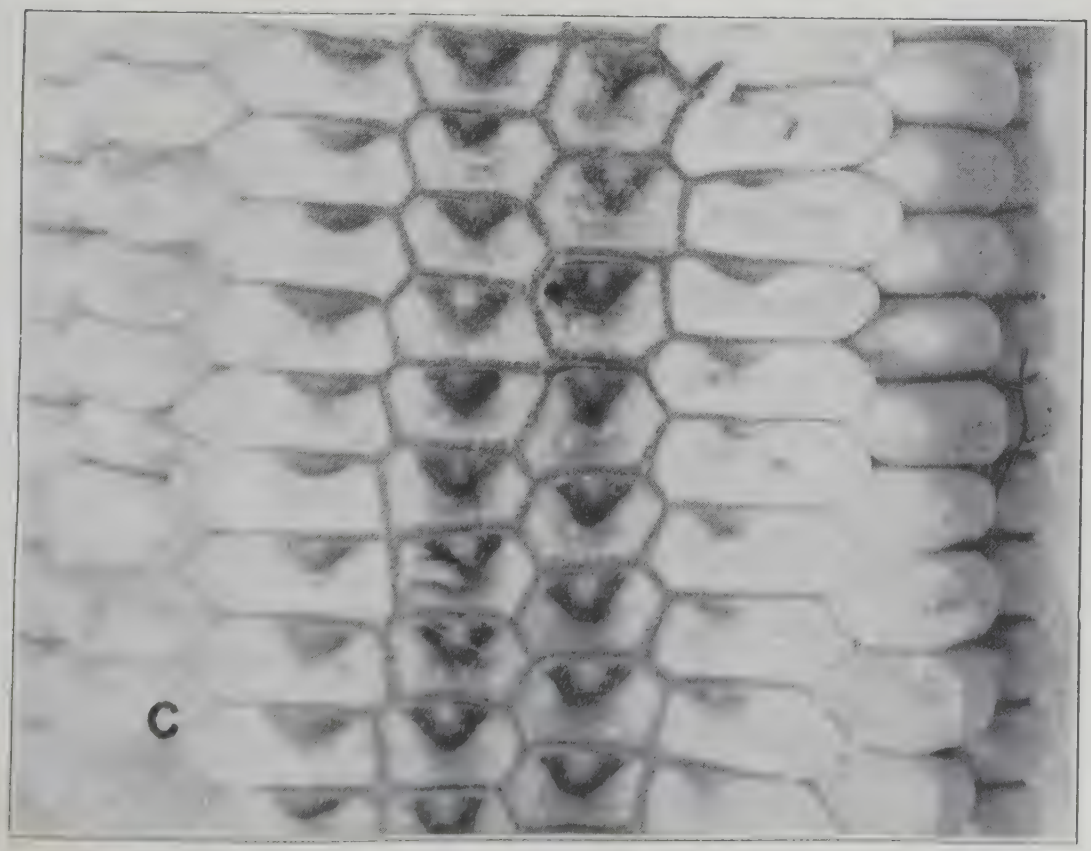
A.—Basal tissues of a stalk taken from the field shown in Plate 15, A. The darkene areas are the vascular plate tissues, zone B, in which the iron and aluminum sal accumulate.

B.—A later stage, showing the basal nodal tissues containing heavy deposits the aluminum and iron compounds. The nodal tissues are disintegrated and mo of the roots have completely rotted.

C.—Transverse section through kernels on an ear of Stowell Evergreen Sweetcor The kernels when tested showed the presence of large quantities of organic iron co pounds. Different ears show variable amounts of iron in them.

A

B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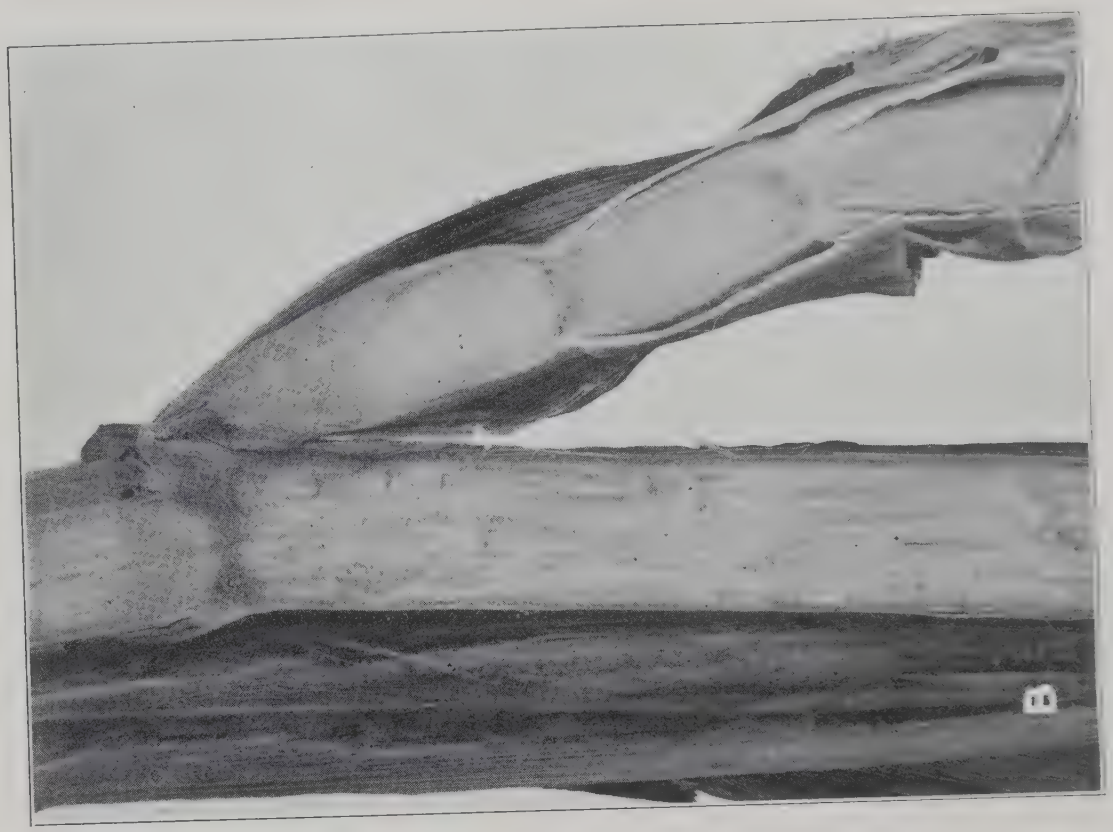
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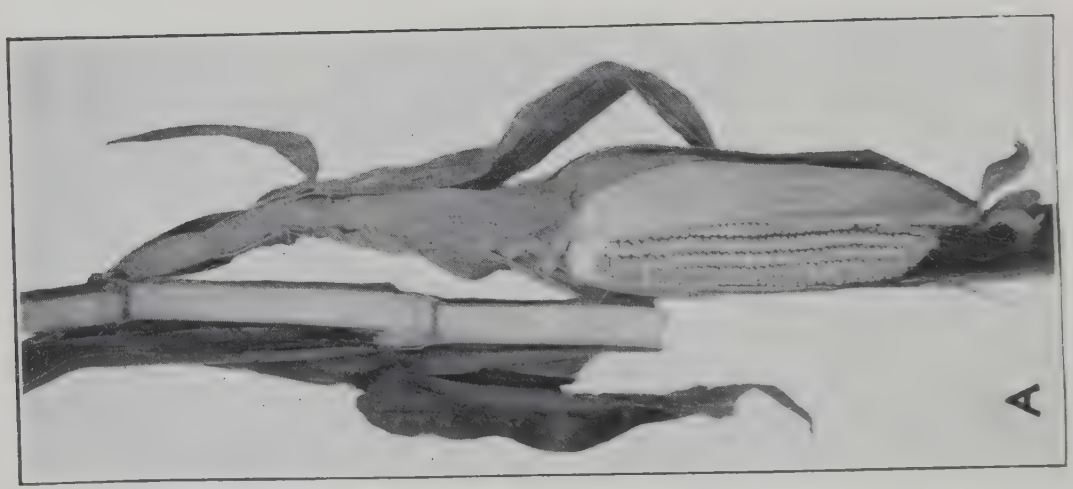

A

PLATE 6

A.—An ear of Reid's Yellow Dent which is in the dough stage of development and is carried on a broken shank. The nodal tissues contained large accumulations of aluminum and iron salts, and the ear-node tissues were disintegrated. Organisms could not be found in the internodal tissues, but local nodal infections at this stage of development of the plant had already occurred.

B.—A close-up of the ear-node tissues. Note the disintegration of the tissues. These weakened tissues afford favorable places for growth of many kinds of organisms.

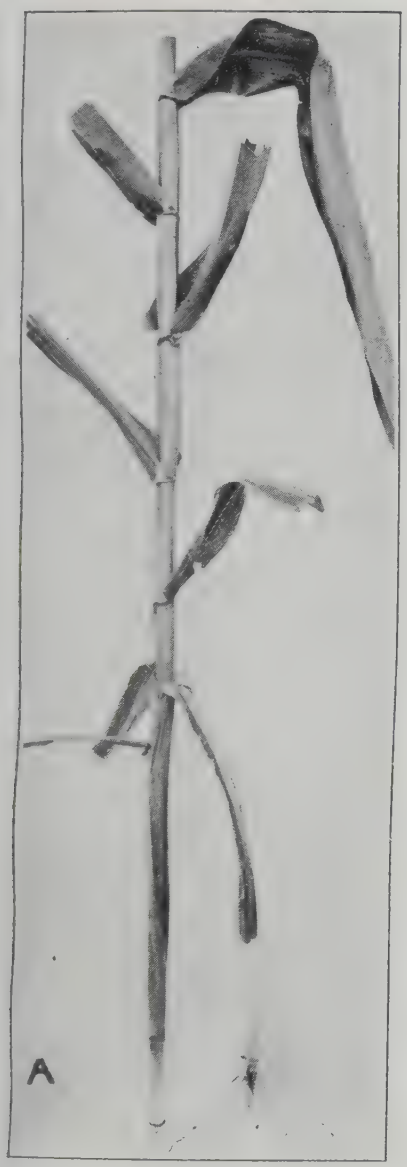
PLATE 7

Views of portions of a diseased corn plant, showing effect of accumulations of aluminum and iron.

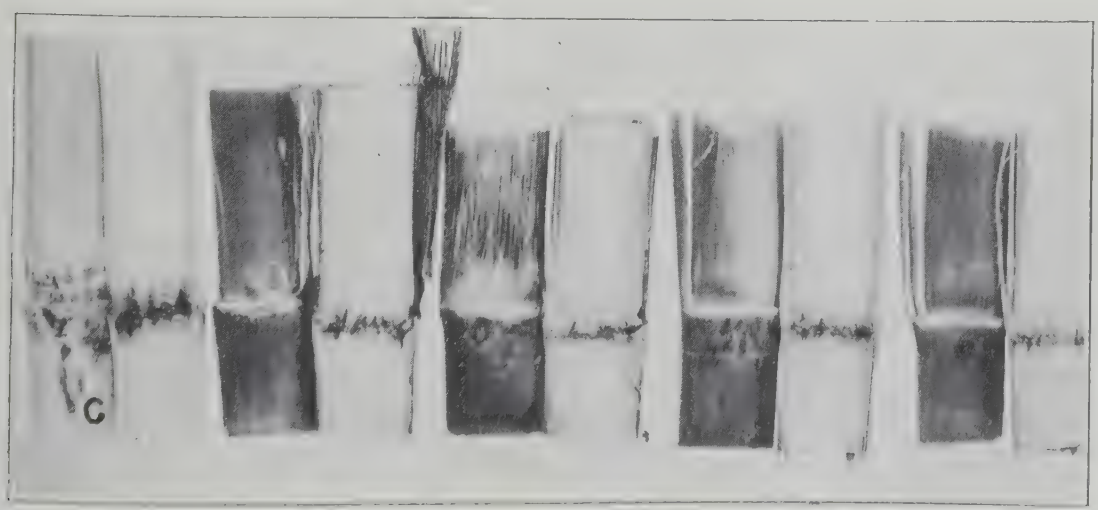
A.—A barren stalk which grew in a hill adjacent to the normal stalk shown in Plate 8, A. The stalk was stripped of its leaves while in the field and then cut longitudinally to be tested for the distribution of the aluminum and iron compounds.

B.—The basal tissues reacted strongly for aluminum and iron compounds. The tissues were dry and badly disintegrated.

C.—The largest accumulations of aluminum and iron compounds are in the lowest nodal plates. This condition and that shown in B are characteristic of many stalks which become rootrotted by the latter part of the growing season.


A

B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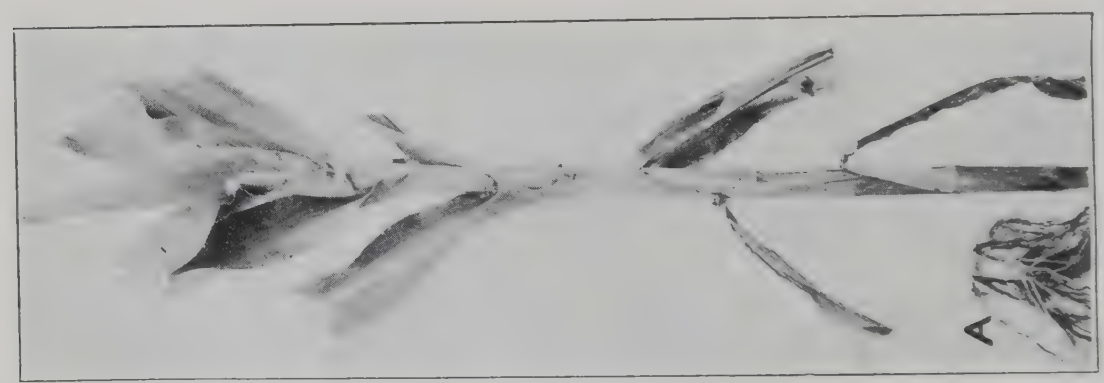

A

PLATE 8

Apparently healthy corn plant which reacted for aluminum and iron.

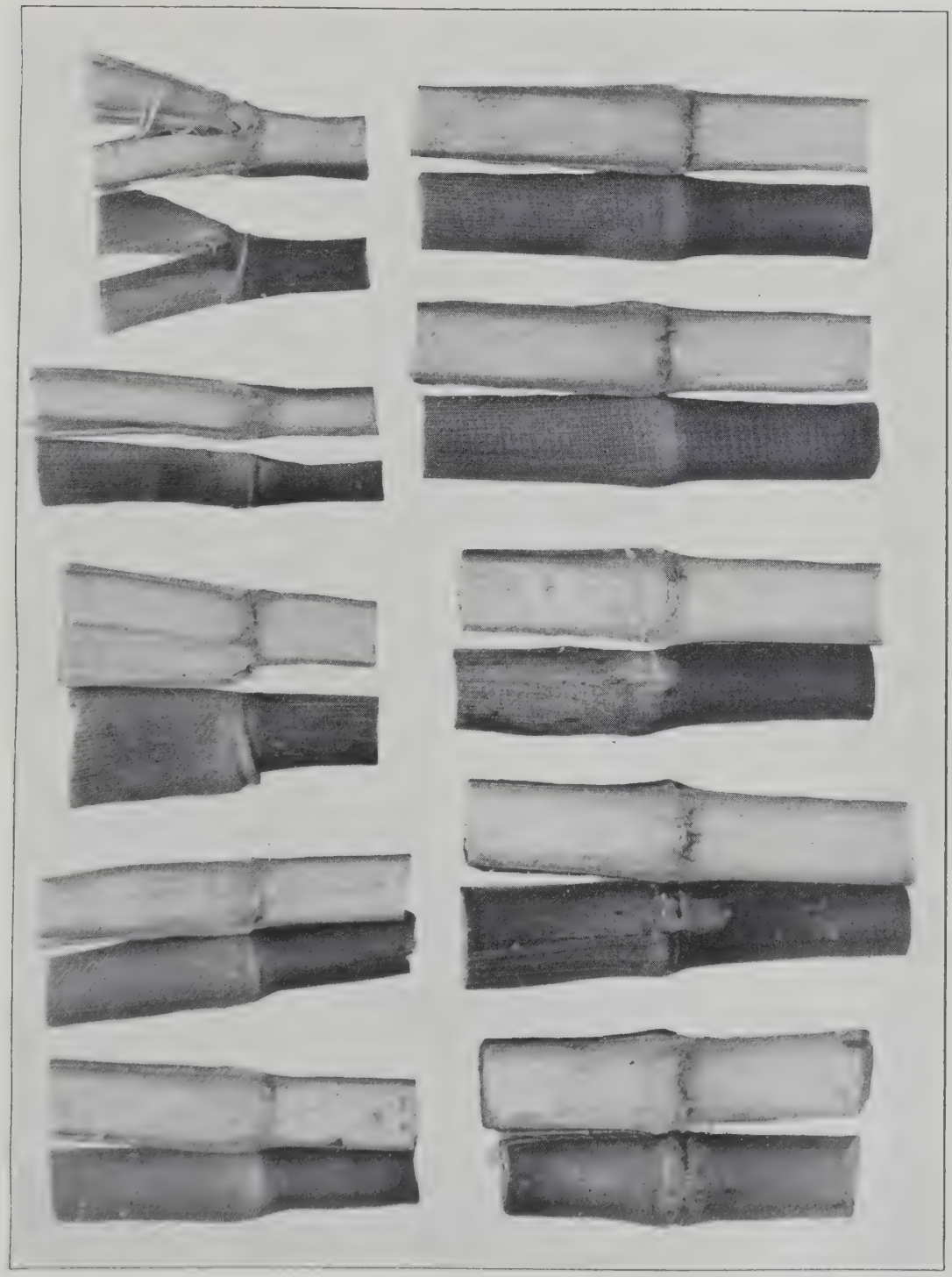
A.—This plant was a normal growing stalk of Reid Yellow Dent. The leaves and the portion of the stalk above the ear were trimmed off in the field. The stalk was sectioned longitudinally in the laboratory and tested for aluminum and iron compounds.

B.—Basal tissues of the stalk shown in A. The vascular bundles which are dark gave reaction for aluminum and iron. Note the lowest roots to the left. They were not functioning even though in moist soil. No rot lesions were found on these roots. This picture was made on the same scale as Plate 7, B.

PLATE 9

Londitudinal sections through the nodes of healthy corn plants.

These nodes were cut from the normal stalk shown in Plate 8, A. The tissues were tested for the presence of aluminum and iron compounds. Note the uniform distribution of the metals in the nodes throughout the stalk. This uniform distribution is characteristic of normal growing plants.



A

B

PLATE 10

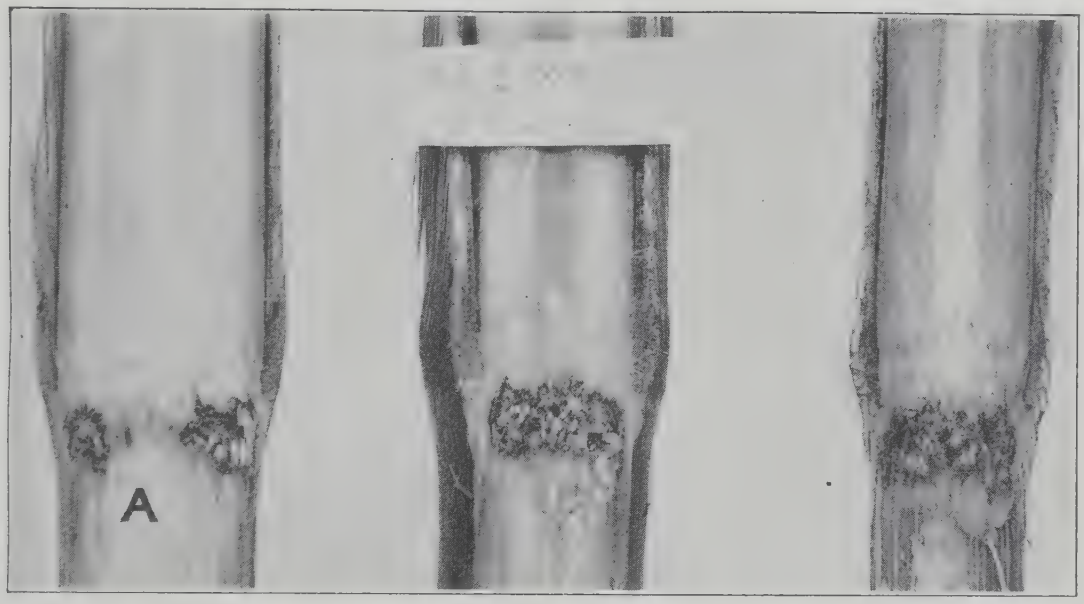
A.—Section through the ear node of a normal appearing stalk of Reid Yellow Dent. Note that the nodal tissues show no disintegration.

B.—Section through an ear node of a stalk of Reid Yellow Dent, showing the disintegration of the nodal tissues due to the effects of aluminum and iron compounds. Fungi, especially *Gibberella saubinetii*, are very frequently isolated from these tissues, but not constantly. *Diplodia zeae* also has been found in stalks in certain fields. This nodal condition, however, has not been found to be associated with any specific organism.

PLATE 11

A.—Effect of aluminum nitrate upon the vascular plate tissue in an injected stalk. An equivalent of 9 mgm. of aluminum caused this injury. The first effect was a browning of the tissues, which was followed by complete disintegration.

B.—The leaf showed first a slight yellowing of the intervascular tissues, giving the appearance of yellow streaks, then a water-soaked appearance, and finally necrosis. The last effect is shown here.


A

B

PLATE 12

A.—This picture shows individual plant differences in resistance to the rootrots. The variety is Johnson County White Dent. Note the scattered, apparently normal stalks. The soil is typical of many of the average acid soils in Indiana. All gradations in growth of the stalks were found in this field; the poorer, stunted stalks are believed to have grown from infected seed. The largest differences in growth and yield between plants grown from infected and disease-free seed seem to occur in soils lacking in some essential nutrient or in which the aluminum and iron compounds occur in large available proportions.

B.—The method used to introduce chemical solutions into the plants. The stalks were first punctured with a sharp cork borer, and the calcium-chlorid tubes were inserted tightly. Measured quantities of the solutions were placed in the tubes for absorption.

PLATE 13

A.—The effect of rootrot in a field of Johnson County White Dent in Shelby County, Ind., in 1918. The stalks grew to good size but fell over before husking time. The soil contains considerable organic matter but was deficient in lime and available phosphates. (Photograph by R. G. East.)

B.—A field of Stowell Evergreen Sweetcorn affected by rootrot at Hoopeston, Ill., in 1919. Many stalks were barren and others had fallen over. The soil was black loam, high in organic matter but deficient in available phosphates.

A

B

1
12
15
18
19
A

2
5
7
9
B

PLATE 14

A.—Pot tests of the soil from the field shown in Plate 4, B, in Shelby County, Ind. Ground limestone at the rate of 3 tons, acid phosphate at the rate of 500 pounds, limestone and acid phosphate at rates of 3 tons and 500 pounds, respectively, and aluminum chlorid at the rate of 500 pounds per acre were the soil treatments. Note the beneficial effects of limestone and of acid phosphate. *Fusarium moniliforme* was isolated from lesions on the roots of plants in each of the pots.

B.—Soil from a field in Hancock County in which the corn was badly rootrotted in 1920. Note that limestone had no effect while acid phosphate was beneficial. These plants also showed rot lesions on the roots. The aluminum treatments cause the plants to become very susceptible to the rots.

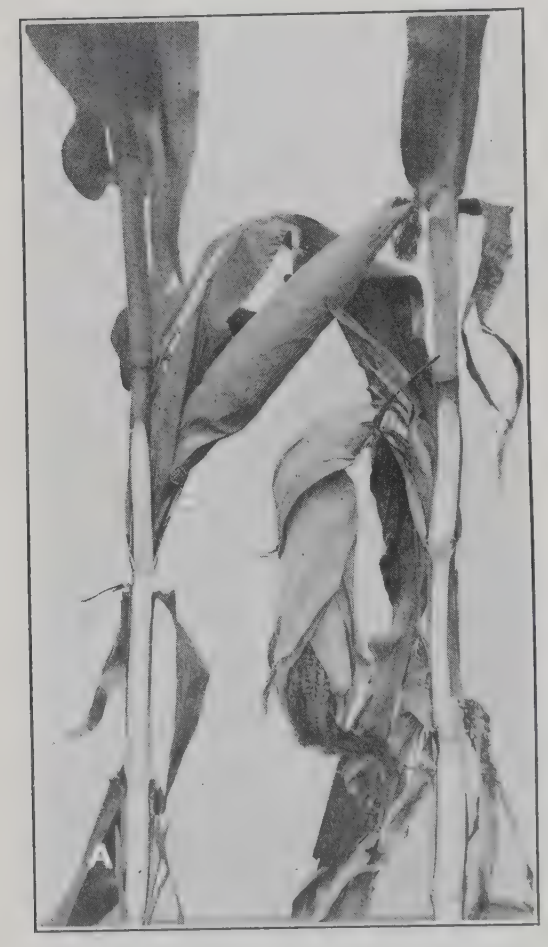
PLATE 15

A.—The control plot in the experimental field of Reid Yellow Dent at Linden, Ind., in 1920. In 1919, rootrot was very severe in this field. Note the stunted growth.

B.—This plot received an application of acid phosphate at the rate of 300 pounds per acre and limestone at the rate of 3 tons per acre. Limestone applied alone at the rate of 3 tons per acre caused injury to the plants in the limestone plot. Pictures A and B were taken on the same day.

A

B


A

B

PLATE 16

A.—Two stalks of Reid Yellow Dent growing in the same hill. The stalk to the right showed large accumulations of aluminum and iron compounds in the nodal tissues, also a yellow-streaked, wilted, and dry condition of the leaves. The stalk died prematurely. The one to the left apparently was in normal condition. The growing conditions had been the same for both stalks. This capacity to absorb available metal salts more rapidly by some stalks than others seems to be an inherited tendency and becomes manifest when the plants grow in certain soils.

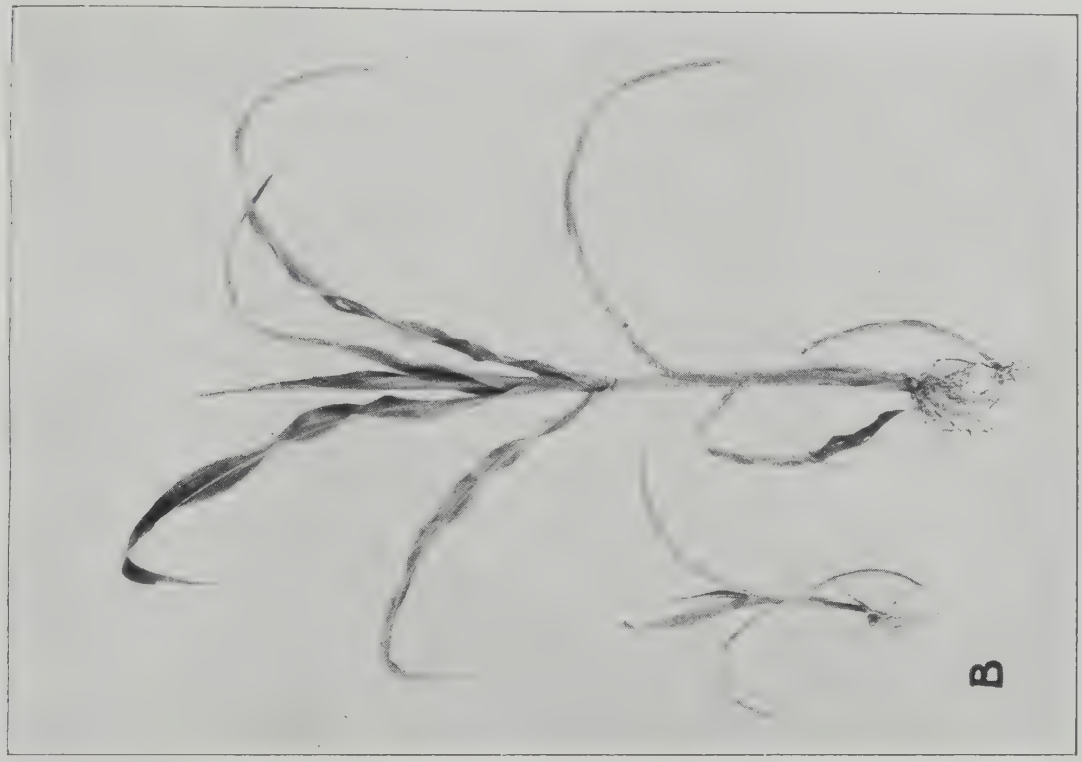
B.—These ears were borne on shanks which broke over prematurely. The ears are starchy, and in this condition they become infected by various organisms, the most common of which are *Fusarium moniliforme* and *Gibberella saubinetii*. They are thus rendered unfit for use as seed. These troubles are most prevalent under conditions of low available phosphates in the soil, or when too late-maturing strains of corn are planted.

PLATE 17

Corn plants showing effect of presence or absence of available iron and aluminum and value of lime as a corrective.

A.—Two plants of Reid Yellow Dent grown from kernels from the same ear. All the plants at one end of the row were stunted. Here the soil gave a test for available iron and aluminum and indicated a lime requirement of 1,100 pounds. The soil at the other end of the ear row gave no test for available aluminum and had an excess lime content of 200 pounds per acre.

B.—Two plants from a row of Country Gentleman Sweetcorn grown close to the row of Reid Yellow Dent shown in A. Note the greater effect of the available aluminum and iron salts upon the sweetcorn plant than upon the dent plant.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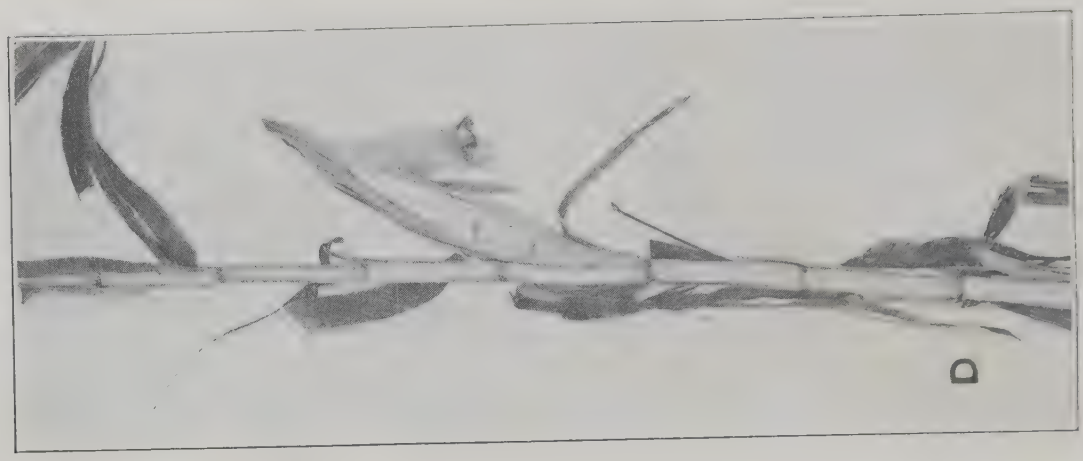
A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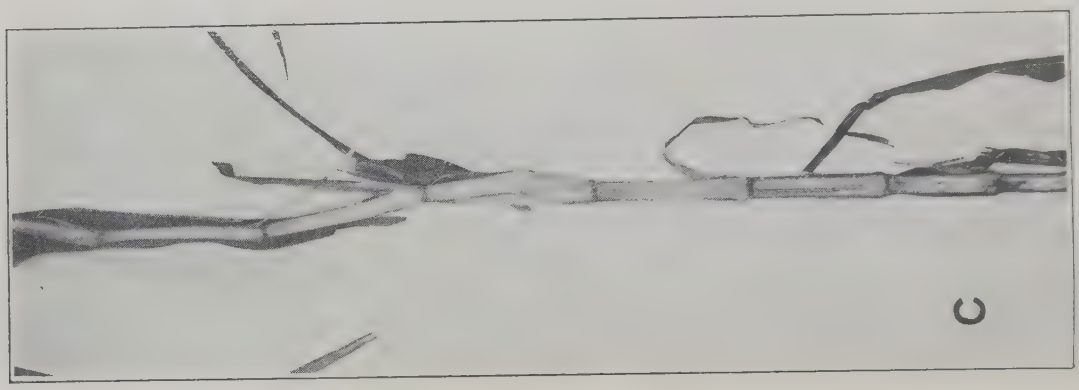
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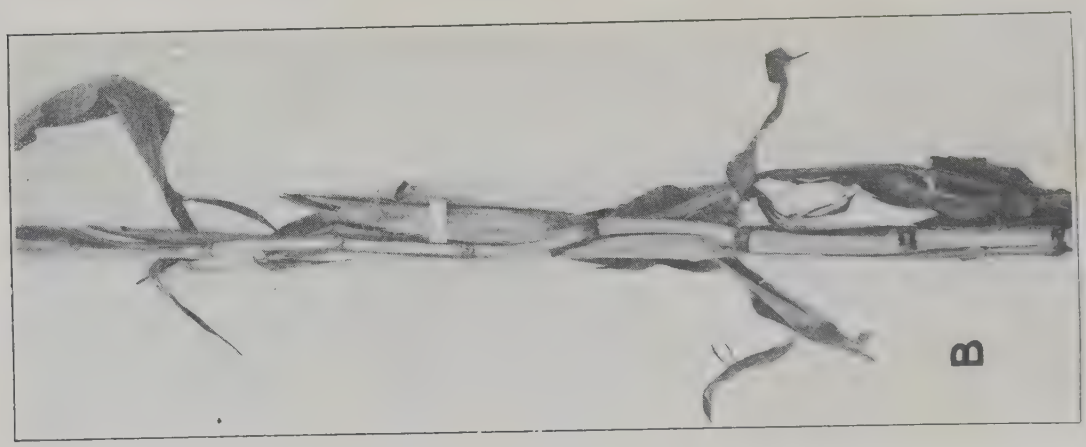
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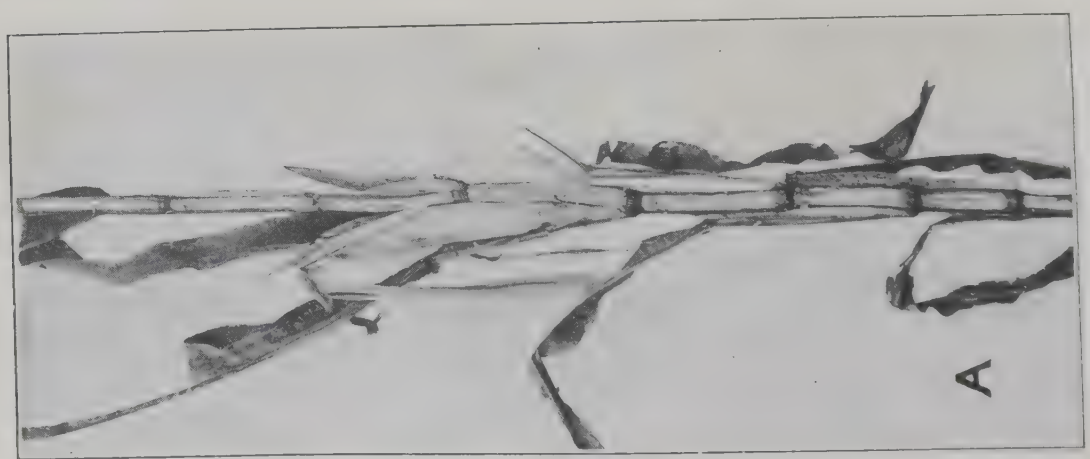
A

PLATE 18

Corn plants showing effect of various chemical compounds on nodal tissues.

A.—Effect of 30 cc. of iron citrate in 1 per cent solution upon the nodal tissues. The vascular plate tissues are darkened, and the leaves are wilted and killed. The plant responded in this manner in three days.

B.—Ferrous sulphate in ½ per cent solution is very toxic. The vascular plate tissues are disintegrated very rapidly. The leaves wilt and the plant turns dark brownish green in color.

C.—Iron nitrate and (D) iron chlorid in 1 per cent solutions do not affect the tissues so severely as the other iron salts. The tissues were not blackened and the accumulations became apparent only upon testing the tissues with a solution of acid thiocyanate of potash.

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PLATE 19

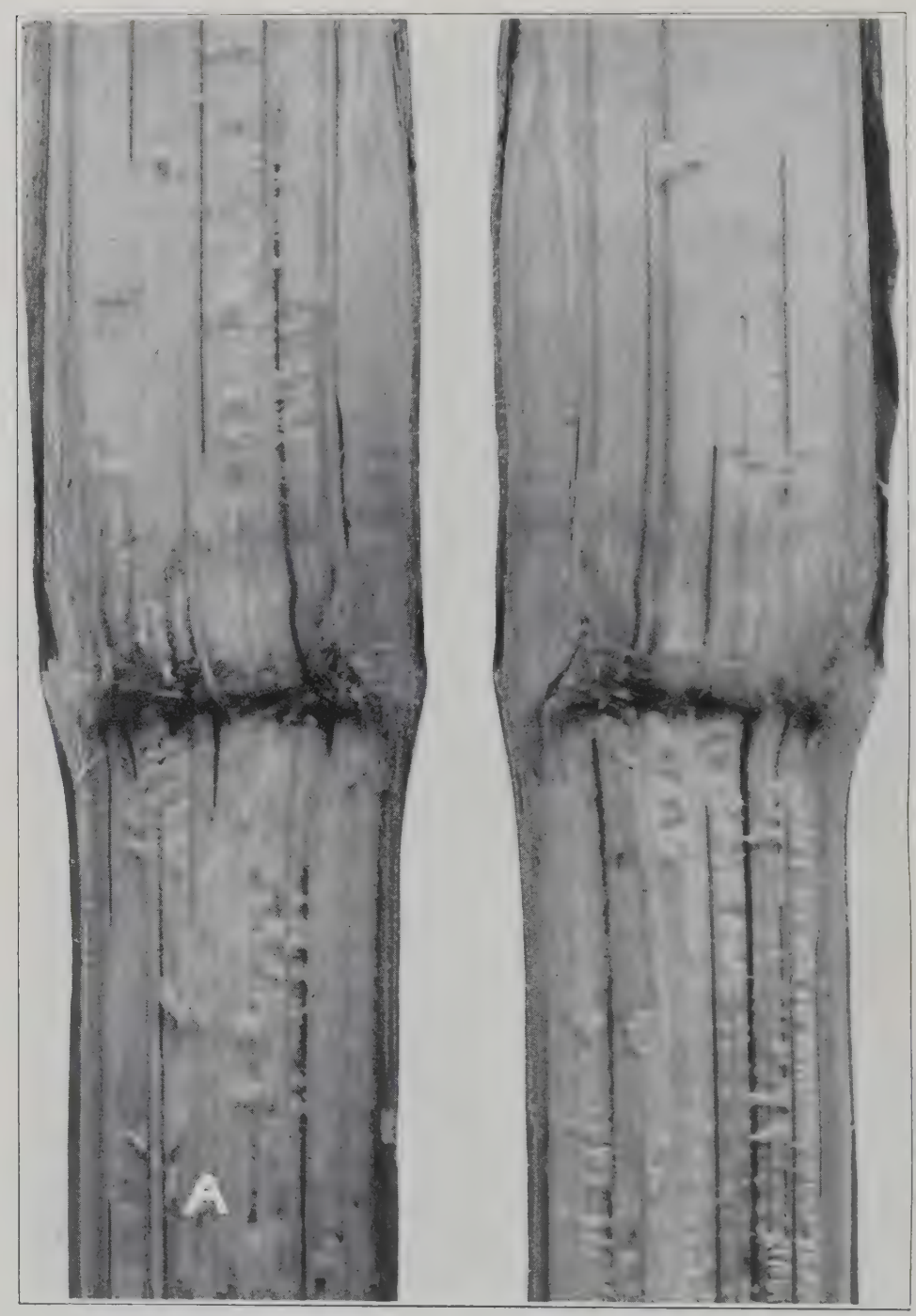
Different parts of a field showing effect of iron and aluminum on corn plants in soil deficient in lime.

A.—Field 6 miles north of La Fayette, Ind. Note the wide range of difference in the growth of the plants in the foreground compared with those beyond. The soil in the foreground gave no reaction for available aluminum and iron salts. The soil in the poorer parts of the field showed available aluminum salts and a lime requirement of 900 pounds per acre.

B.—Part of the field in A where abundant blighting of the plants occurred. Note the markedly reduced stand and weak plants. Seedling-blight is very prevalent under such soil conditions.

A

B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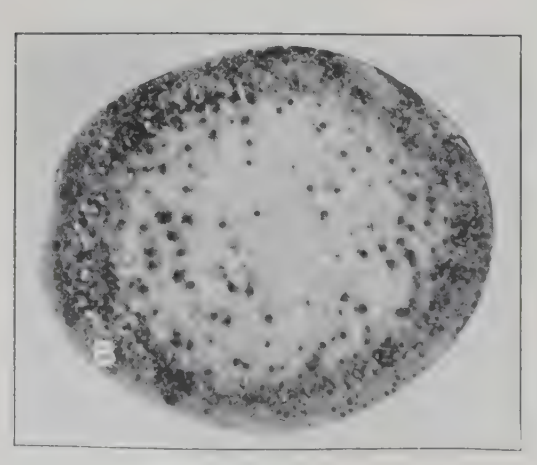
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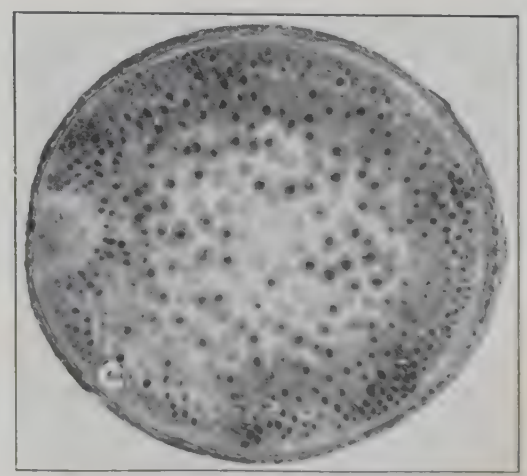
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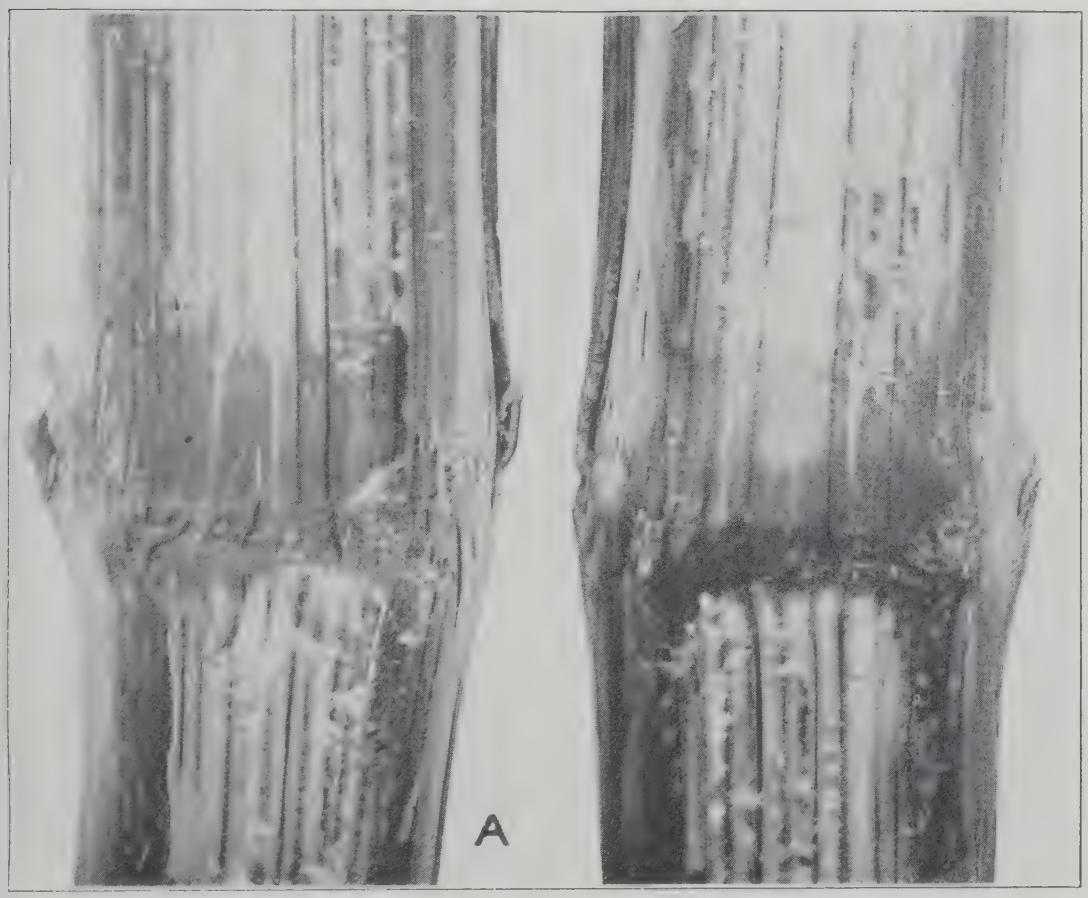
PLATE 20

A.—Longitudinal section through a node of a stalk showing symptoms of rootrots. The darkened vascular bundles are stained by a dilute solution of methylene blue which was absorbed by the plant. Compare the number of bundles which are functioning with the number shown in Plate 21. The nonfunctioning bundles have the largest accumulations of iron and aluminum in the zone B region.

B, C.—Relative numbers of functioning vascular bundles in transverse sections of diseased stalks. B shows the internode section and C a section through zone A.

PLATE 21

The picture in A shows a longitudinal section through the node of a normal stalk. The dark bundles were stained by a dilute solution of methylene blue which was absorbed by the plant. The bundles were practically all functioning, as is observed in the transverse sections through the stalk shown in B. The same treatment was given to this stalk as to those shown in Plate 20.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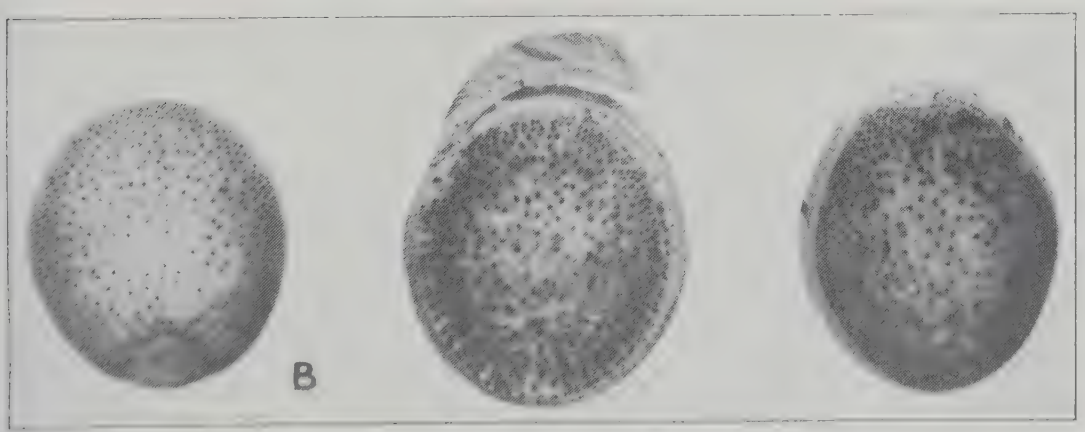
B

PRODUCTION OF A GROWTH-PROMOTING SUBSTANCE BY AZOTOBACTER [1]

By O. W. HUNTER

Associate Bacteriologist, Kansas Agricultural Experiment Station

It is maintained by many investigators that the animal cell is incapable of synthesizing vitamines and that such cells are thus required to obtain their food accessories from the vegetable kingdom. If this is true, it can logically be asked, do plants need such growth promoting factors? If so, do they synthesize them or from what source are they obtained?

Bottomley's (*2*) [2] experiments on peat led him to state that the growth of plants require food accessories or auximones. He grew aerobic soil organisms in peat for several days. This was then sterilized, inoculated with Azotobacter and incubated. As a result there were produced in the peat substances which greatly stimulated plant growth. Similar results were obtained by Mockenridge (*4*) and Jones (*3*).

The application of the vitamine theory to microbial nutrition is now prevalent. While there has been a large amount of attention focused upon the need of food accessory factors for the normal growth of microorganisms, but little thought has been placed upon the vitamine content of microorganisms or their ability to synthesize such, regardless of the fact that one of the most abundant known supplies of the so-called water-soluble B vitamine is found in a one-celled organism, the yeast. This vitamine content of yeast was first reported by Funk (*1*).

Pacini and Russell (*5*), from their experiments concluded that *Bacillus typhosus* Eberth-Gaffky can manufacture vitamines and can likewise stimulate growth of the animal cell. They grew *B. typhosus* in a synthetic medium, the bacterial cells from which were collected and extracted with alcohol. The alcoholic extract was evaporated down to dryness and the residue dissolved in water. This extract was used in their feeding experiments on white rats.

Recently much attention has been directed toward the use of yeast for the quantitative measurement of the vitamine content of test substances, the principle of which is dependent upon the assumption that the yeast needs a food accessory factor for its growth in an inorganic synthetic medium. This accessory factor is considered identical with the water-soluble B vitamine.

Experiments to determine the reliability of the test appear to have brought forth some interesting facts. This dependence of yeasts upon the antineuritic vitamine is doubted by MacDonald and McCullom (*7*).

[1] Accepted for publication Jan. 16, 1922. Contribution No. 39 from the bacteriological laboratories of the Kansas Agricultural Experiment Station, reporting in part the results of project No. 138.

[2] Reference is made by number (italic) to "Literature cited," p. 831.

They cultivated yeast in a synthetic medium containing no possible source of vitamine and obtained a good growth. They concluded that the yeast must either synthesize its own vitamine or grow without such "bios." Nelson, Fulmer, and Cessna (*6*) likewise grew yeast in a synthetic medium and concluded that yeast could synthesize its own vitamine.

The controversy over the vitamine needs of yeast stimulated the author's interest in the Azotobacter cell. The ability of this organism to grow rapidly and vigorously in a synthetic medium containing no nitrogen is familiar. Such a medium contains no possible source of vitamines, if care is exercised in its preparation. Media thus prepared in this laboratory have always afforded a vigorous growth of Azotobacter when placed under favorable conditions for growth. It is assumed, therefore, that the Azotobacter organism is not dependent upon such growth-promoting factors for development or that it is capable of synthesizing vitamines for its needs.

Under these conditions it would be of interest to learn whether this growth resulting from Azotobacter development contained any vitamines. Their presence would thus indicate the ability of Azotobacter to synthesize growth promoting substances.

Preliminary feeding experiments on white rats, with a diet in which the water-soluble B vitamine was replaced by dried Azotobacter cells, revealed the possible presence of a growth promoting substance. In this experiment the Azotobacter was grown on dextrose Ashby agar placed in large wooden trays to give a large surface exposure. The growth was scraped from the surface of the agar after two days' incubation at 30° C. and air dried.

Three white rats were fed on the following diet:

	Per cent.
Starch	58
Casein	20
Lard	10
Butter	5
Ash	3
Agar	2
Azotobacter	2

The beneficial effects of the feed are shown in figure 1:

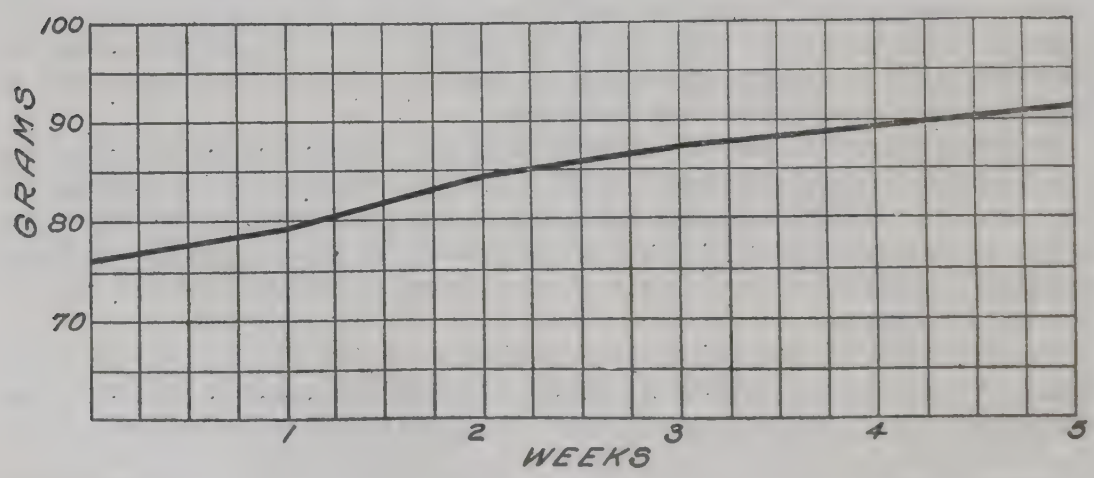

FIG. 1.—Average gain for three rats fed Azotobacter. Control rats died.

Three rats fed on the same diet minus the Azotobactor failed to gain in weight, developed the characteristic sore eyes, and soon died.

These results indicated the possibility of the presence of a food accessory factor for rats fed on a diet deficient in water-soluble B. However, needed

improvement in the medium used and in the method of cultivation were desired for further investigation. This was accomplished by devising a means for cultivating the Azotobacter rapidly in a large volume of liquid culture medium.

A description of the method of procedure and experimental data follows.

MEDIUM

To insure the absence of any known food accessory compound, chemically pure chemicals were employed in the preparation of the synthetic medium. All media were made up with a reaction of P_H 7.0 to 7.4 and were thoroughly sterilized at 20 pounds pressure for 30 minutes.

The composition of the medium was as follows:

Distilled water	1,000.00 cc.
Potassium acid phosphate	.50 gm.
Magnesium sulphate	.20 gm.
Sodium chlorid	.20 gm.
Dextrose	10.00 gm.

STARTERS

Starters of pure Azotobacter cultures were made by inoculating flasks containing about 300 cc. of the dextrose media with a portion of the emulsion from a young dextrose Ashby slant culture. This was thoroughly aerated for two days at a temperature of 30° C. Several strains of Azotobacter cultures have been used in these experiments. All cultures were isolated in this laboratory from different samples of soil. No attempt has been made to differentiate the cultures as to species. Pigment production was common in all cultures used.

It was aimed to use only pure cultures of Azotobacter, and repeated streakings upon dextrose Ashby agar plates were made to insure freedom from contamination. Frequent morphological examinations were made to insure purity of the culutres.

METHOD OF CULTIVATION

A rapid multiplication of Azotobacter cells was encouraged by vigorous aeration of the liquid cultures. Bottles containing 2,000 cc. of sterile media were inoculated with 10 to 20 cc. of the Azotobacter starter. These were placed in the incubator, temperature of 30° C., and aerated by bubbling air vigorously and continually through the medium. The air was passed through the cultures by attaching the bottle equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the cultures were reduced to a minimum by filtering the air, first through a sterile cotton filter and then by washing in two or three flasks of sterile water. The air was thus filtered through cotton and rinsed in water before entering the culture media. From 12 to 24 of these bottles were aerated at the same time. A vigorous growth was produced within two to four days. If the culture is pure and no contamination occurs, the reaction of the media should remain close to the reaction of the media before inoculation.

It is not believed that the inoculum supplies accessory food factors for stimulating the growth of Azotobacter (in this synthetic medium). Ten flasks containing 200 cc. of the media were inoculated, respectively,

in duplicate with 2 cc., 1 cc., 0.5 cc., 0.1 cc., and 0.01 cc. of a young starter. All cultures were aerated at 30° C. for four days. Sugar determinations were made at the end of two and four days. All cultures exhibited a heavy growth within two days. The sugar analysis at this time showed the flasks to contain the following amounts of dextrose:

	Gm. dextrose per 100 cc.
Flasks with 2 cc. inoculum	0.60
Flasks with 1 cc. inoculum	.53
Flasks with 0.5 cc. inoculum	.60
Flasks with 0.1 cc. inoculum	.48
Flasks with 0.01 cc. inoculum	.33

The cultures gave only a trace of dextrose at the end of four days' incubation. The growth at this period was more vigorous in all cultures than the preceding one. It does not appear from these results that the growth of Azotobacter in such a synthetic medium could be attributed to stimulating factors carried over with the inoculum. The Azotobacter growth in the following experiments was collected by centrifuging the liquid culture in a Sharples Laboratory Super-Centrifuge. The residue was recovered and air dried.

FEEDING EXPERIMENTS UPON WHITE RATS

The presence of a growth-promoting substance in this dried Azotobacter was tested upon white rats. The dried Azotobacter was added to a diet deficient in water-soluble B vitamine. The composition of the feed used was as follows:

	Per cent.
Starch	58
Extracted casein	10
Tankage	10
Ash	3
Agar	2
Butter	10
Lard	5
Azotobacter	2

On April 28 six white rats were placed in individual pens and fed until May 24 on a regular stock food. Upon this date three of the rats were placed upon the experimental feed and three upon the same feed, except that Fleischmann's yeast was substituted for the Azotobacter.

The stimulating effect of each test substance is demonstrated by the curves in figure 2, plotted from the data obtained. It will be observed that during the intervals between the second and fourth weeks but little gain was recorded. This is attributed to the ash content of the feed. A new portion of feed containing new ash was prepared at this time. The rats fed on the Azotobacter feed show a greater comparative growth than the rats fed on the yeast diet. The average weights of the three rats fed on the Azotobacter feed is compared with the average weights of the three fed on the yeast food by the curves plotted in figure 2. The results from this experiment indicate that Azotobacter is fully as good as baker's yeast, if not better, as a growth-promoting substance for white rats fed on a diet deficient in water-soluble B.

EXPERIMENTS WITH PIGEONS

The curative effect of Azotobacter upon pigeons affected with polyneuritis was noted. A pen of pigeons was started on a feed of polished rice and water July 14. When pronounced symptoms of polyneuritis

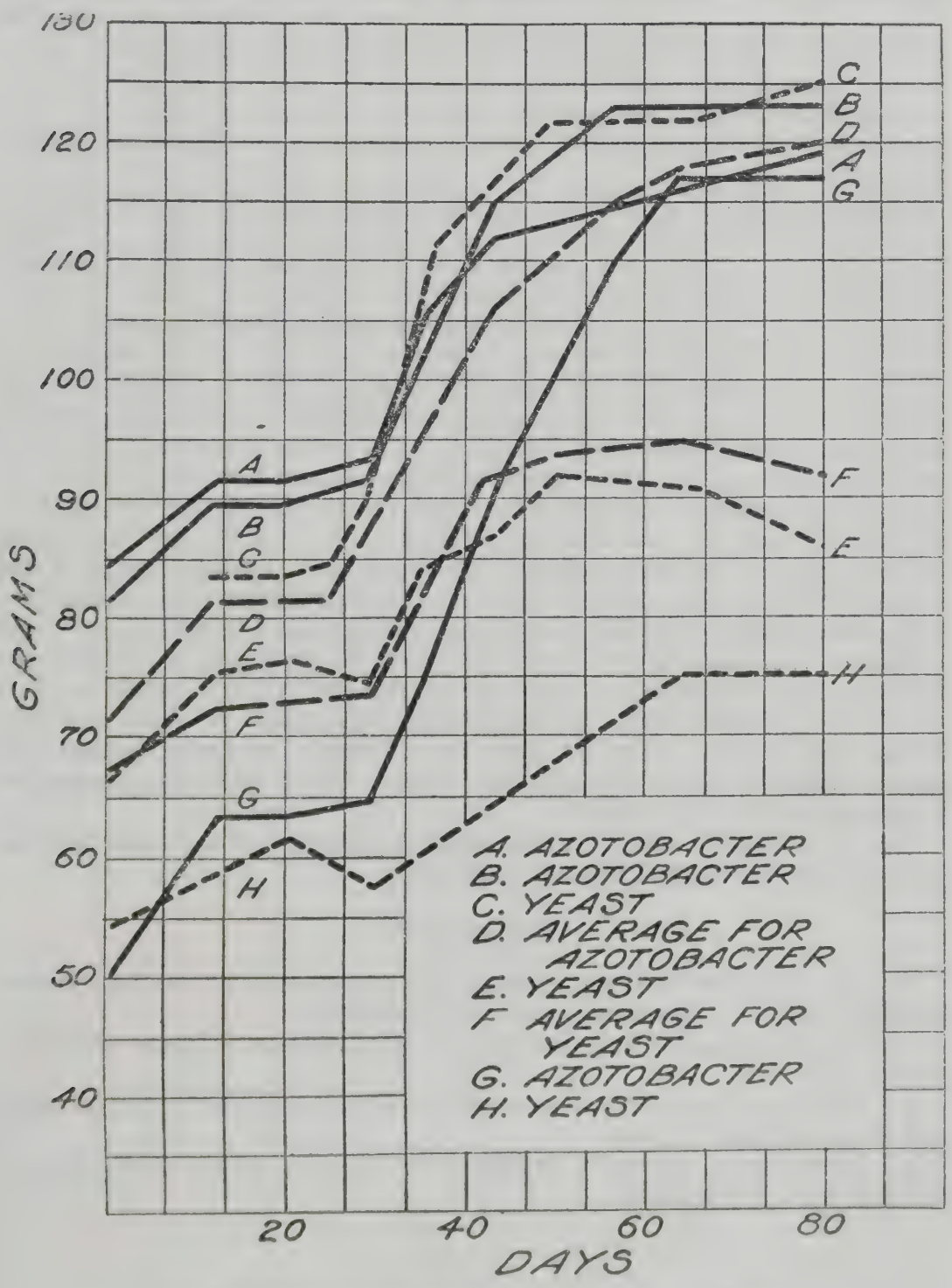

FIG. 2.—Influence of Azotobacter and yeast upon the growth of rats.

were observed, the sick pigeon was fed by hand, portions of the dried Azotobacter. The treated birds were left on the rice diet throughout the experiment. The record of observation follows:

Pigeon 3743 showed symptoms of polyneuritis on July 25. It grew daily worse and upon July 28 was exceedingly lame and unable to stand

on its feet. Upon this date it received 1 gm. of Azotobacter and on the following day it was up on its feet but still lame. On July 31 it was fed another gram. Its recovery was practically complete, with the exception of a slight lameness in one leg. Symptoms of polyneuritis were again evident on August 6, by lameness and inability to stand on its feet. It was fed 1 gm. of Azotobacter in the morning of this date. Marked improvement was shown by evening, and the next morning it was up on its feet feeding about the pen. On August 8 the pigeon appeared in normal health, with the exception of a slight lameness in one leg. The experiment was discontinued on this date and no further observations were made.

Very pronounced symptoms of polyneuritis appeared in pigeon 3742 on July 4. It was given on this date 1 gm. of Azotobacter. The next day it was on its feet and was given 0.5 gm. Its recovery was seemingly complete. On July 14 it again developed "limberneck" and leg weakness and died that night. This time it received no Azotobacter.

Pigeon 3740 developed very pronounced symptoms of polyneuritis on July 27 in the form of "limberneck" and inability to stand or walk. One gram of Azotobacter was given it on this date. Much improvement was noted the following day. On July 29 it received another gram and the following day 0.5 gm. Marked improvement followed, and to all outward appearances recovery was complete. It developed leg weakness but no "limberneck" the morning of August 6. At this time it received another gram of Azotobacter, and this dose was repeated again in the evening. The following morning it was up and feeding about the pen. No further observations were made, as the experiment was discontinued.

Pigeon 3741 developed typical polyneuritis July 25, exhibiting the characteristic "limberneck" and leg weakness. It was given on July 27, 1 gm. of Azotobacter. Improvement was noted the following day. It was fed another gram on July 29, although its improvement was marked, and on July 30 it received 0.5 gm. To all general appearances it was normal the next day.

That Azotobacter exerts a pronounced curative effect upon pigeons affected with polyneuritis seems conclusive.

CONCLUSIONS

(1) If a growth-promoting substance is a requirement for Azotobacter development, it is capable of manufacturing such.

(2) The Azotobacter can synthesize a food accessory factor similar to water-soluble B.

(3) This food accessory factor stimulated a greater net gain in white rats than did baker's yeast.

(4) Azotobacter exerts a pronounced curative effect upon pigeons affected with polyneuritis.

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SCLEROTINIA CARUNCULOIDES, THE CAUSE OF A SERIOUS DISEASE OF THE MULBERRY (MORUS ALBA)[1]

By EUGENE A. SIEGLER, *Assistant Pathologist, Office of Fruit-Disease Investigations* and ANNA E. JENKINS, *Assistant Mycologist, Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture*

In 1903, Orton[2] described a disease of the mulberry characterized by the "peculiarly enlarged portions of the aggregated fruit." The disease was said to occur in Georgia, Alabama, and Mississippi. Dr. M. B. Waite, Pathologist in Charge, Office of Fruit Disease Investigations, Bureau of Plant Industry, states that specimens of mulberry fruits showing this condition have been received from various southern States by the United States Department of Agriculture a number of times during the past 20 years. Taubenhaus[3] reports the occurrence of this disease in Texas, where it is known under the name of "popcorn" disease of mulberry.

In July, 1920, specimens of diseased mulberry fruits (*Morus alba* L.) were received from Scranton, S. C. Specimens of this material show the greatly enlarged condition of the ovary (Pl. 1, A), and the calyx lobes are small and nonsucculent instead of being fleshy as in the normal fruits. Upon microscopical examination the ovaries are found to be entirely filled with mycelium and all traces of host tissue are lost. The hyphae are compact, producing a typical sclerotium, and preventing the formation or further development of drupelets. A layer of sporogenous hyphae completely envelops this sclerotium and produces small hyaline spores in a compact palisade within the ovary wall (Pl. 1, B). The spores, presumably microconidia, are often exuded in immense numbers and collect in what resembles a waxy mass on the outside of the ovary wall (Pl. 1, C, *a*). It is interesting to note the occurrence of microconidia on the diseased fruit, as no reference to microconidia of species of Sclerotinia occurring other than on culture media has been found in the literature. This disease undoubtedly is the same as that reported by Orton.[4]

The nature of the disease led us to suspect that we had either a species of Sphacelia, or, more probably, the microconidial stage of the genus Sclerotinia. It was thought that infection probably occurred at the blooming period of the host and resulted in the formation of such a sclerotium. Accordingly on March 18, 1921, an inspection was made of the planting in South Carolina from which diseased specimens had been received in 1920. At that time apothecia (Pl. 2, A) of a fungus belonging to the genus Sclerotinia were found in large numbers on the ground beneath the infected trees. The apothecia were attached to the infected

[1] Accepted for publication Oct. 28, 1921. The technical description from page 835 of this paper was printed in Science, vol. 55, no. 1422, p. 353, March 31, 1922.

[2] ORTON, W. A. ON A FUNGUS DISEASE OF THE MULBERRY FRUIT. (Abstract.) *In* Exp. Sta. Rec., v. 14, no. 6, p. 531–532. 1903.

[3] TAUBENHAUS, J. J. ON A PECULIAR DISEASE OF MULBERRY FRUIT. *In* Nature Study Rev., v. 17, no. 7, p. 282–285, 3 fig. 1921.

[4] ORTON, W. A. OP. CIT.

seeds (really ovaries) which were buried at an average depth of ½ inch in the soil. The ascospores were found to be mature on this date and capable of germinating in tap water. A microscopical examination of the blossoms showed practically 100 per cent infection at this time. An opportunity was not afforded to inoculate healthy blooms with ascospores.

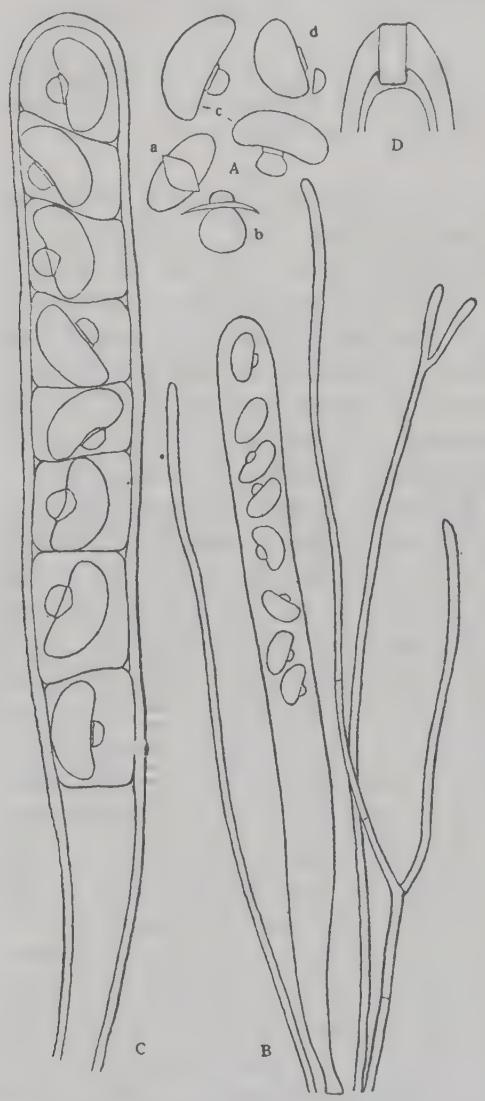

FIG. 1.—*Sclerotinia carunculoides:* Asci, ascospores, and paraphyses. A, Ascospores with bodies adjoining the concave surfaces; *a*, view from above showing rhombic shape of body adjacent to the spore; *b*, transverse section showing crescent shape of the body adjacent to spore; *c*, longitudinal section of spore, showing location of hemispherical body; *d*, spore with hemispherical body dislodged. ×1,600. B, Ascus, ascospores, and paraphyses. ×840. C. Ascus, showing ascospores surrounded by gelatinous membrane producing a cross-walled effect. ×1,600. D, tip of ascus. ×1,600.

Specimens of young blooms not less than 8 feet above the nearest group of apothecia were collected on March 18, placed in a killing solution, embedded in paraffin, and later sectioned. Upon microscopical examination of the prepared mounts typical ascospores (fig. 1, A) were readily found on the pistils. The foregoing observations constitute sufficient evidence to warrant belief in the pathogenicity of the fungus.

No record of a similar disease of mulberry in this country has been found. Two somewhat similar diseases due to *Sclerotinia shiraiana* P. Henn. and *Microglossum shiraianum* P. Henn. have been reported in Japan. In a letter of June 20, 1921, Dr. Shirai, of the Tokyo Imperial University, states that these fungi attack—

> mulberry fruits and form sclerotia just in the same manner ... and occur on the tree at the same time.

He also states that these two fungi can not be distinguished by the forms of the sclerotia but that the fruiting bodies are entirely different. Material of *S. shiraiana* was kindly sent by Dr. Shirai for comparison with the fungus collected in South Carolina. The latter differs in many respects from *S. shiraiana* and especially in its effect on the fruit and in the size and form of the ascospores. In *S. shiraiana* the entire aggregate fruit is transformed into a sclerotium (Pl. 2, B, *a*, *b*), while in our fungus the multiple fruit breaks up and the sclerotia are formed from individual druplets (Pl. 2, A, *b*). There is also greater variation in the size of the druplets in the American material as compared with the specimens from Japan (Pl. 2, B, *c*). A Botrytis stage develops in the fruits attacked by *S. shiraiana*, while in this country there is no record of a Botrytis on mulberry fruit. However, it is possible that a conidial form other than the microconidial may be found in this country.

In connection with the differences in the size and form of the ascospores, it should be noted that the spores of *Sclerotinia shiraiana* are elliptical and are, according to comparison with material received from

Dr. Shirai, slightly larger than the spores of our fungus. The spores of the latter are reniform, and attached to the concave surface of each spore are peculiar bodies (fig. 1, A) which are striking structures morphologically. Viewed under low magnification these bodies resemble a caruncle, (fig. 1, B). Under higher magnification they are seen to be composed of two parts, one a body adjacent to the spore and more or less rhombic in shape as seen from above (fig. 1, A, *a*) and crescent-shaped (fig. 1, A, *b*) in transverse section of the spore, the second a body adjoining the first and more or less hemispherical (fig. 1, A, *c*). No description of similar bodies has been found in the literature on Sclerotinia or on closely related genera. The presence of these caruncle-like bodies made possible the identification of the ascospores on the pistils. Further studies are being made on the nature of these structures. Stained preparations show the spores to be surrounded by a gelatinous-like substance which appears to be broken up into segments in the ascus, so that the ascus has somewhat the appearance of being marked by transverse septa (fig. 1, C). Somewhat similar appearances are shown by Woronin [5] on Plate 10, figures 21 to 23, of his drawings of *Sclerotinia megalospora* Wor. and by Boudier [6] on plate 318 of his drawings of *Peziza cucullata* Boud.

As stated above, this fungus differs morphologically from *Sclerotinia shiraiana*, which produces a somewhat similar but distinct disease in Japan; it also appears distinct from any other described species. The name, *Spermatomyces mori*, gen. nov., sp. nov., which was suggested by Orton (l. c.),[7] referred to the microconidial stage, and since no technical description was published, it must be considered a *nomen nudum*. The fungus is described as follows:

Sclerotinia carunculoides, n.sp.[8]

Apothecia one to several from a single sclerotium, disc cupulate to subcupulate, 4 to 12 mm. in diameter, inside snuff-brown,[9] outside Prout's brown; stalk cylindrical, flexuous, smooth, attenuated downward, 15 to 42 mm. in length, reaching a diameter of 1.5 mm., color Prout's brown; asci cylindrical to cylindro-clavate, 104 to 123μ by 6.4 to 8μ, average 117 by 7μ, 8-spored; ascospores uniseriate, reniform, hyaline, 6.4 to 9.6μ by 2.4 to 4μ, average 7.6 by 3.1 μ, with two bodies on the concave surface: namely, a body more or less rhombic in shape as seen from above, 2 by 4 μ, and adjoining it, a more or less hemispherical body 3 μ in its longest diameter; paraphyses filiform to cylindro-clavate, simple or branched, septate or nonseptate, 94 to 128 μ by 1.8 to 2 μ; microconidia hyaline, subglobose, 2 to 4 μ by 2 to 3.2 μ, average 2.8 by 2.5 μ; sclerotia black, fairly regular, subspherical with depressed surfaces.

On fruits of cultivated *Morus alba*. Type material collected at Scranton, S. C., U. S. A., March, 1921. Specimens have been deposited in the Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

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[5] WORONIN, Michael. ÜBER DIE SKLEROTIENKRANKHEIT DER VACCINIEEN-BEEREN. ENTWICKELUNGS GESCHICHTE DER DIESE KRANKHEIT VERURSACHENDEN SCLEROTINIEN. Mém. Acad. Imp. Sci. St. Pétersb. sér. 7, t. 36, no. 6, 49 p., 10 col. pl. St. Pétersbourg, 1888.

[6] BOUDIER, Émile. ICONES MYCOLOGICAE OU ICONOGRAPHIE DES CHAMPIGNONS DE FRANCE PRINCIPALEMENT DISCOMYCETES.....t. 2, pl. 318 (col.), t. 4, p. 177. Paris. 1905-1910.

[7] ORTON, W. A. OP. CIT.

[8] *Sclerotinia carunculoides*, sp. nov.

Ascomatibus ex uno sclerotio solitariis vel pluribus exorientibus, discis cupulatis vel subcupulatis, 4–12 mm. diam., intus sternutamento-brunneis, extus "Prout's"-brunneis; stipitibus cylindraceis, flexuosis, glabris, deorsum attenuatis, 15–42 mm. longis, usque 1.5 mm. diam., "Prout's" brunneis; ascis, cylindraceis vel cylindro-clavatis, 104–123×6.4–8 μ, medio numero 117×7 μ, octosporis; ascosporis uniseriatis, reniformibus, hyalinis, 6.4–9.6×2.4–4 μ, medio numero 7.6×3.1 μ, faciebus superis duo corpusculis praeditis: videlicet corpusculo plano, nonnihil rhombio desuper viso 2×4 μ; et hoc attingente altero corpusculo plus minusve hemisphaerico, longissimo diametro 3 μ; paraphysibus filiformibus vel cylindro-clavatis, simplicibus vel ramosis, septatis vel non-septatis, 94–128×1.8–2 μ; microconidiis hyalinis, subglobosis, 2–4×2–3.2 μ, medio numero 2.8×2.5 μ, e hyphis nigris, mediocriter regularibus, sphaeroideis, superficiebus depressis.

In fructibus cultis Mori albae, Scranton, South Carolina, U. S. A.

[9] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. IV, 43 p., 53 col. pl. Washington, D. C. 1912.

SUMMARY

Sclerotinia carunculoides, n. sp., is described as causing a disease of fruits of cultivated mulberry (*Morus alba*) completely destroying them as an edible fruit.

The disease is known to occur in South Carolina and has been reported from other southern States.

Ascospores of this fungus have been found on blooms which were collected not less than 8 feet above the nearest group of apothecia.

The occurrence of the microcondidial form of the fungus in the diseased fruit is noted.

The most striking feature which distinguishes this fungus from others of the same group is the presence of a prominent caruncle-like body on the concave surface of the ascospore.

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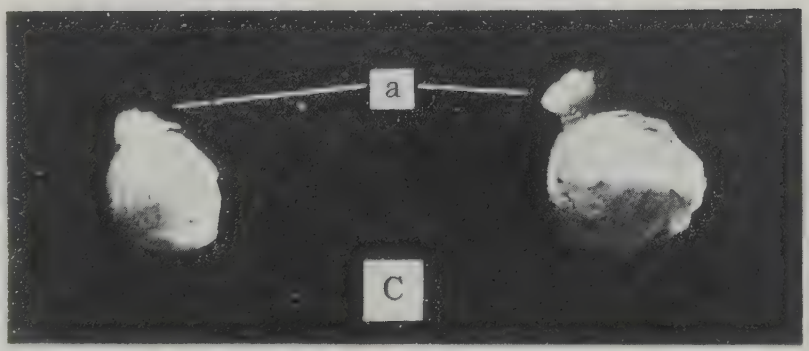
PLATE 1

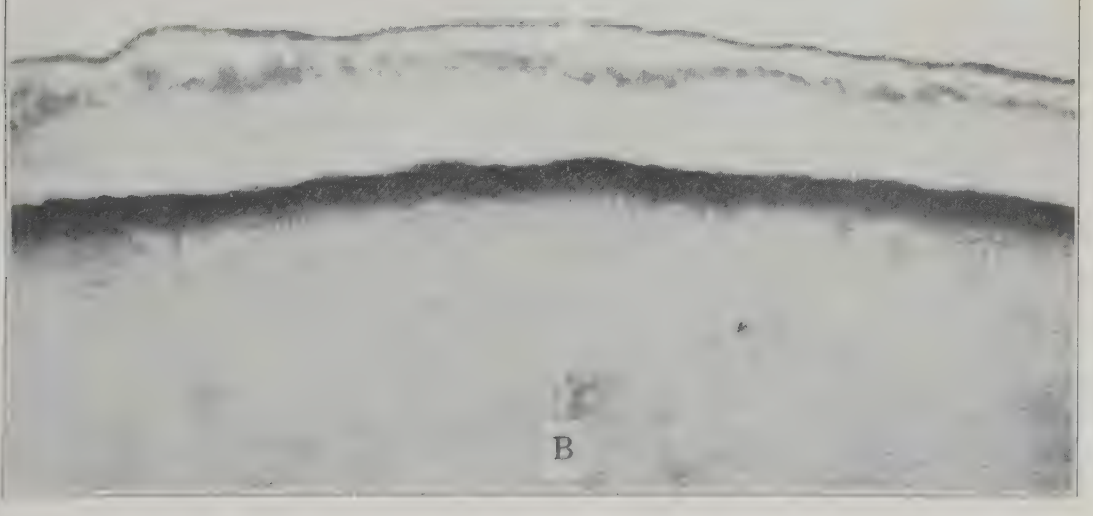
A.—Diseased fruits of *Morus alba*, showing the greatly enlarged condition of the ovaries. × 1 1/3.

B.—Photomicrograph of section of infected ovary, showing the sclerotium and layer of microconidia, indicated by arrow. × 75.

C.—Infected ovaries (*a*), mass of microconidia on the outside. × 3.

A





B

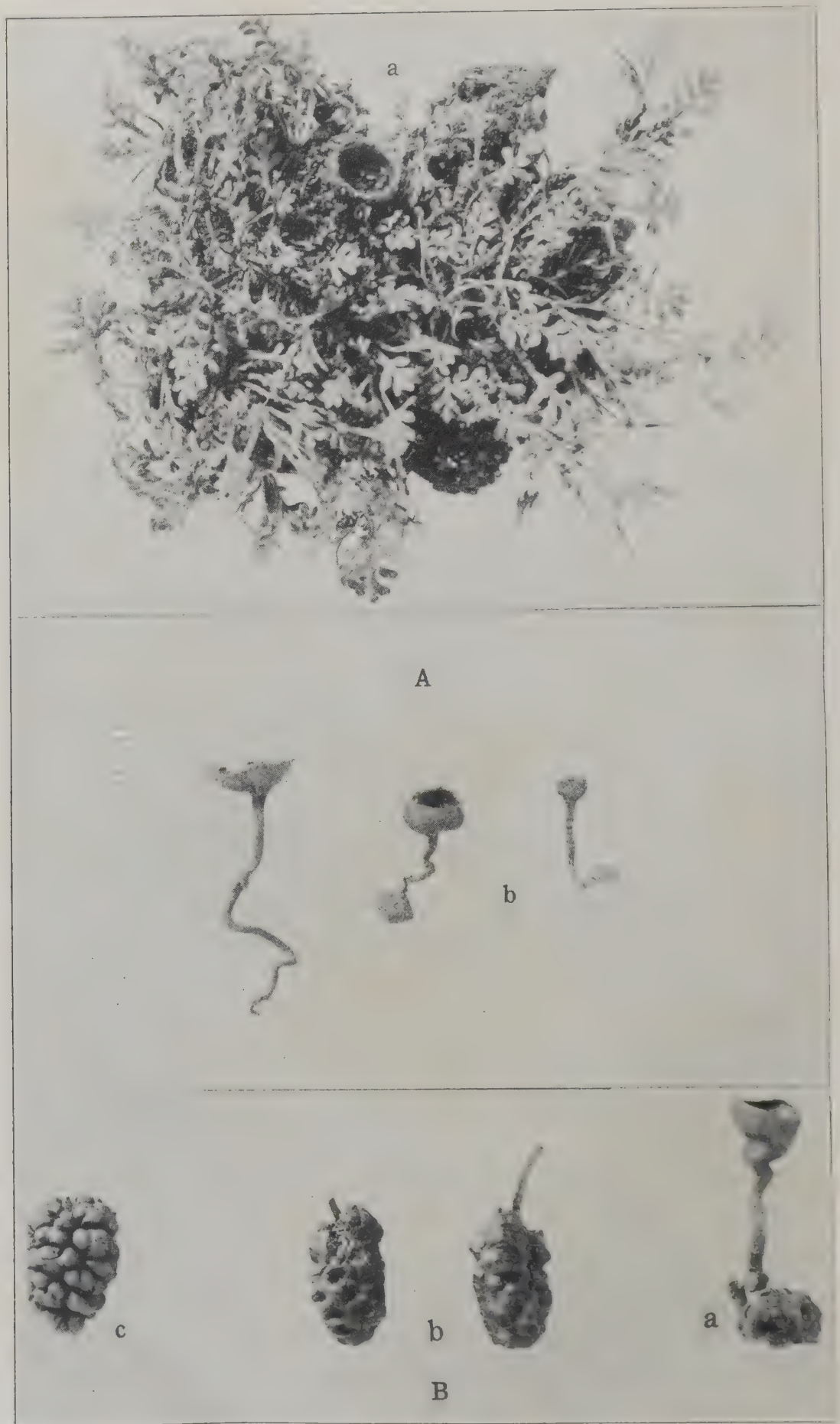

a
A
b
c
b
a
B

PLATE 2

A.—Apothecia of *Sclerotinia carunculoides*: (*a*) in situ, (*b*) attached to old seed (sclerotia). Natural size.

B.—*Sclerotinia shiraiana*: (*a*) apothecium arising from sclerotium composed of entire fruit, (*b*) sclerotia, (*c*) infected fruit, showing the comparative regularity in size of the druplets. × 1 1/4.

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INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF THE SEEDLING-BLIGHT OF WHEAT AND CORN CAUSED BY GIBBERELLA SAUBINETII[1]

By JAMES G. DICKSON, *Associate Professor of Plant Pathology, University of Wisconsin, and Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Various seedling-blights, rootrots, and damping-off troubles of plants have long been associated with unfavorable soil and weather conditions. The soil-inhabiting organisms which cause this group of relatively obscure diseases usually become aggressive parasites only when the seedling or plant is weakened through its inability to react favorably to the environment. Plant pathologists are continually being reminded of the important correlation between climatic conditions and the occurrence of plant diseases. The fact is emphasized by Duggar (*10*),[2] Smith (*28, p. 36 et seq.*), and Jones (*21*) that the most important, most complex problems for long-time research include the critical study of the relation of environment to parasitism.

From the field standpoint, this problem was well illustrated in Illinois during the fall of 1919. The first part of the period for sowing winter wheat was hot and dry, followed later by cool, wet weather. The early sowings of wheat were made, therefore, in a comparatively warm and dry soil, whereas the later sowings were made in a cool and moist soil. In many of the early sown fields, the seedling-blight caused by the conidial stage of *Gibberella saubinetii* (Mont.) Sacc. destroyed more than half of the young seedlings. Chiefly on account of these ravages, together with Hessian fly injury, the stand and vigor were so greatly reduced that a number of such early sown fields were resown the following spring. In contrast to this, in the fields sown late in the autumn it was almost impossible to find a blighted seedling, even in the worst-infested fields.

These conditions are analogous to those affecting the development of cabbage yellows as noted by Gilman (*11*). Experiments show conclusively that the soil organism *Fusarium conglutinans* Wollenw. is capable of inducing cabbage yellows only at soil temperatures of 17° C. or above. Tisdale found that flaxwilt (*Fusarium lini* Bolley) developed

[1] Accepted for publication May 10, 1922. The investigations upon which this paper is based were conducted cooperatively by the Office of Cereal Investigations of the Bureau of Plant Industry and the Wisconsin Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," pp. 869–870.

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only at soil temperatures above 16°. These observations and experiments tend to confirm Wollenweber's (*34*) generalization that the root-invading Fusariums are warm-soil organisms.

The Fusarium seedling-blight of wheat and other cereals (*Gibberella saubinetii*) has proved to be well adapted to a critical study of the relation of environment to parasitism, as the same parasite is able to attack a number of different host plants, especially wheat and other small grains and corn. The field observations on winter wheat, mentioned previously, indicate that soil temperature and soil moisture are important factors in the development of the disease. As a result of the suggestions from these field observations and the apparent importance of the diseases on both wheat and corn, investigations were undertaken to determine the relation of soil temperature and soil moisture to the development of seedling-blight of wheat and corn caused by *G. saubinetii*. The responses of the two hosts, wheat and corn, were so markedly different that the studies are being continued with the view of determining the physiological and chemical responses which determine host susceptibility. Although the results here presented are not complete, it is believed that they will serve to show the relation of temperature and moisture to the development of the blights caused by this one organism.

SEEDLING-BLIGHT OF WHEAT

The seedling-blight of wheat was not connected with the headblight or scab described by Smith (*29*), Weed (*33*), Chester (*8*), Arthur (*3*), and Hickman (*16*), until Selby and Manns (*27*) described the diseased seedlings obtained by sowing scabbed kernels. The seedling-blight has been mentioned since, and its close association with unfavorable weather conditions has been suggested by Bolley (*6*, *7*), Schaffnit (*25*), Akerman (*1*), Atanasoff (*4*), and others. This disease is prevalent in the winter-wheat areas of the Mississippi Valley. The losses from this stage of the disease vary greatly from year to year, depending upon climatic conditions.

The symptoms of the seedling-blight caused by *Gibberella saubinetii* vary, depending upon the portion of the seedling attacked and the environing conditions during the development of the blight. In case of severe attacks many of the germinating seedlings may be killed before they emerge, which results in reduced stand. When the attacks are less severe the seedlings emerge and are weakened or killed, according to the severity of the attack. Such seedlings, when carefully removed from the soil before disintegration, show all gradations of attack (Pl. 1 and 2). In many cases the developing embryo is invaded and killed before either roots or plumules elongate (Pl. 3, B). The embryo as well as the invaded portions of the kernel become reddish brown to carmine red as the soil-moisture condition varies from dry to wet. The fungus growth tends to accumulate over the surface of the infected tissues, causing the soil to cling tightly to the diseased parts.

In other instances, the plumule is blighted when less than 1 cm. long, the root development continuing, however, for some time afterwards (Pl. 1, *b*, and 3). Often the roots develop to a length of 5 to 10 cm. before they are invaded by the fungus. The invasion usually occurs by the parasite growing from the invaded endosperm into the proximal portions of the roots, after which the entire root is invaded and rotted. Other seedlings do not succumb to the attack until the first-leaf or often the

second-leaf stage. The attacked plants first become dwarfed and chlorotic, then wilt (Pl. 1, *c*), and finally fall over and become overrun by the parasite (Pl. 3, B). This latter stage is a typical wilt of the young plant. The old seed and the portion of the stem below ground develop the characteristic reddish brown color. Usually perithecia form gregariously on the culm near the soil line. Still other seedlings partly succumb to a root attack. In many cases all of the roots may be badly rotted and the plant considerably dwarfed. Such plants, when weather conditions are favorable for the development of the seedling, may send out new roots from the first node and entirely or partially recover (Pl. 2, *a*, and 3, A).

In many instances the reddish brown lesions occur only on the coleoptile without penetrating deeper into the tissue. The symptoms under these conditions are very similar to the same type of lesion produced by light attacks of Helminthosporium; the color in the former lesion, however, generally is slightly lighter.

SEEDLING-BLIGHT OF CORN

The relationship between seedling-blight and rootrot of corn and wheat scab caused by *Gibberella saubinetii* was earlier suggested by Arthur (*3*) and later proved by Hoffer, Johnson, and Atanasoff (*17*). This organism causes heavy losses in corn through the central and upper Mississippi Valley. In 1920, fields were reduced 10 to 25 per cent where corn was planted on fields in which the wheat crop, heavily infected with scab, had been plowed under the previous fall, while field inoculation experiments the same year demonstrated yield reductions of 10 to 30 per cent. Although the distribution and prevalence of this parasite on corn has not been definitely determined, yet it is evident that the organism is capable of causing heavy losses in the corn crop as well as in wheat.

The symptoms of the seedling-blight of corn caused by *Gibberella saubinetii* vary, as do those of wheat, depending upon the severity of attack, the age of the seedling, and soil conditions. As in the case of wheat, the corn seedling may be attacked in various stages, the type of infection being similar to that in wheat (Pl. 4, A, B, C). The lesions are more definite on the corn seedling, however, and usually extend from the kernel into the tap root and lateral roots as well as into the mesocotyl.[3] The invaded areas are at first light brown, water-soaked regions, soon turning reddish brown. The kernel turns reddish brown to carmine red, depending upon soil moisture conditions. The type and color of the lesion, as well as the color of the infected kernel, are distinctly different from similar infections produced by *Helminthosporium* sp. or *Diplodia zeae* (Schw.) Lev. The badly infected seedlings become yellow and wilt down in a manner very similar to that described for wheat. Many of the plants partially recover by sending out new roots and make a fair growth, although a rather high percentage of such plants fail to produce marketable ears.

The symptoms of the seedling-blights of corn caused by *Gibberella saubinetii* are in general very similar, the chief difference being the development of a more definite lesion on the larger stem and roots of corn. The symptoms on both hosts are variable, depending as they do upon many varying factors.

[3] The term "mesocotyl," as used in this paper, follows previous usage for corn, applying to the structure between the attachment of the scutellum and the base of the coleoptile.

The period of severe infection in both wheat and corn is usually restricted to the seedling stage. Few new infections occur after the seedling has reached the fourth-leaf stage. Occasionally, however, under weather conditions favorable for the development of the disease new root lesions may develop, which tend to reduce the vigor of the plant but rarely develop sufficiently to kill it.

LIFE CYCLE OF THE ORGANISM IN RELATION TO THE PRODUCTION OF SEEDLING-BLIGHT

The seedling-blight of wheat and corn develops from two chief sources; first, scabbed or infected seed and, second, infested soil. Floral infections in wheat develop during moist, warm weather and result in blighted or scabbed kernels within the infected spikelets. The mycelium hibernates in or on the scabbed kernels, many of which show no marked external symptoms until the seed is sown, when the parasite develops in the young seedling. Seed infection also occurs in corn. The mycelium hibernates in the seed and develops in the young seedling in the spring, following germination.

In addition to seed infection, the organism develops as a saprophyte on decaying crop refuse near or on the surface of the soil. It becomes aggressive as a parasite only when the seedlings are weakened by unfavorable environment.

EXPERIMENTAL METHODS

CULTURE METHODS

The temperature and moisture studies were conducted under controlled conditions in the greenhouse as well as under field conditions. Pure-line Marquis wheat (Wis. Ped. No. 50) was used in all of the spring-wheat studies and pure-line Turkey wheat (Wis. Ped. No. 2) was employed in the winter-wheat studies. Specially selected ears of Funk Ninety-Day yellow dent and Golden Glow yellow dent were used in all of the experiments. Only kernels free from disease and treated with mercuric chlorid 1 to 1,000, followed by rinsing, were used for inoculation and controls. In addition, naturally infected, scabbed wheat kernels were used in several experiments in comparison with artificially inoculated seed.

In the greenhouse the soil-temperature experiments were conducted in the Wisconsin soil-temperature tanks, considerably modified from those described by Jones (*21*) and Johnson and Hartman (*20*). The tanks, as constructed on the unit plan, consisted of the insulated water bath equipped with a rigid cover through which the soil containers were suspended in the water bath below.

Containers of two different sizes were used. A can 6 inches in diameter by 12 inches deep was used for most of the seedling studies, and a larger can 12 inches in diameter by 20 inches deep for the plant studies. In addition to being utilized as soil containers these larger cans were fitted with covers and used for culture chambers. These, together with the Altmann incubator, were used in the studies on the relation of temperature to the development of the fungus.

The water within the tank was heated by an electric heater in a watertight copper container submerged at the bottom of the tank. The heater was controlled by a mercury thermostat set in the water bath and an

ordinary telegraph relay. The temperatures below greenhouse temperature were maintained by running a stream of cold water into the tank, lowering the temperature of the water slightly below that desired, and then heating the water to the temperature required by the thermostatically controlled electric heater.

By means of this equipment it was possible to maintain fairly constant soil temperatures in the containers as well as constant air temperatures within the chambers consisting of the submerged cans. Representative soil-temperature records are compared with the air temperature in the greenhouse in figure 1. Soil-temperature readings, 2 inches below the surface, were recorded several times each day throughout the duration of all the experiments, and in many instances soil-thermograph records were obtained. The mean soil temperature for the duration of the experiments is given in each series of data presented. The Altmann incubator was operated at intervals ranging from about 4° to 30° C. The tanks were operated at regular intervals of 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C., although the mean given in the table in connection therewith may deviate slightly from these temperatures.

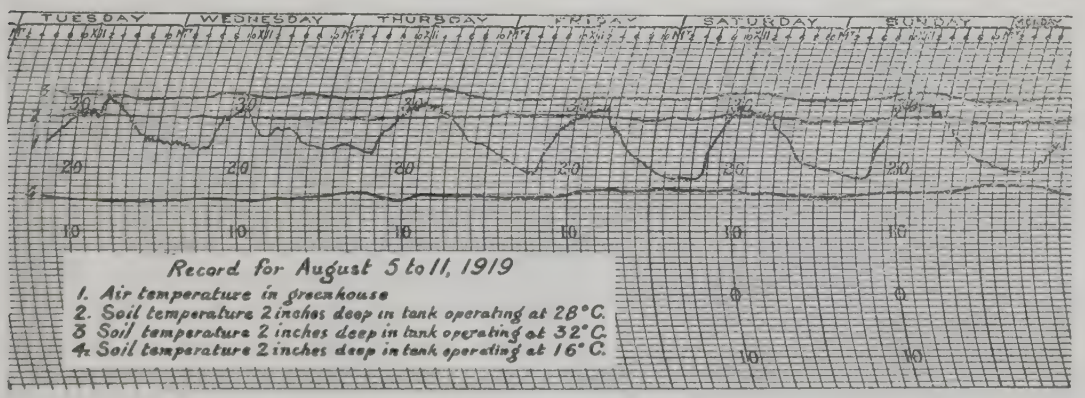

TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
SATURDAY
SUNDAY
Record for August 5 to 11, 1919
1. Air temperature in greenhouse
2. Soil temperature 2 inches deep in tank operating at 28° C.
3. Soil temperature 2 inches deep in tank operating at 32° C.
4. Soil temperature 2 inches deep in tank operating at 16° C.

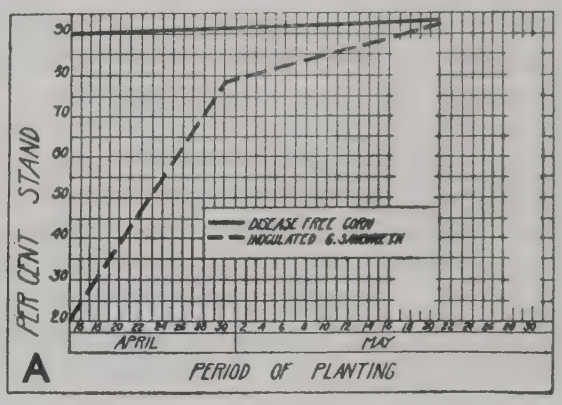
FIG. 1.—Graphic record of greenhouse air temperature and of soil temperature in three different tanks used in the experiments.

The approximate quantity of soil necessary to fill all the cans used in the series was thoroughly screened and mixed, after which the moisture content and moisture-holding capacity of the soil were determined. Sufficient water was then added to bring the moisture up to the desired point of saturation, after which the soil was again thoroughly mixed. After standing covered for a day, the soil was mixed repeatedly and potted, a weighed amount being placed in each can. The cans were equipped with a small inverted flowerpot placed in the soil at about the level of the seed bed. This pot was connected with the surface by a short glass tube fitted with a plug to prevent air circulation. The fully equipped container was then weighed and the weight recorded, after which the can was placed in the temperature tank.

Soil moisture was kept as nearly constant as possible during the progress of the experiments by daily weighing and adding water to restore the original weight. Evaporation from the soil surface was prevented by a dust mulch or coarse sand on the surface. In this way the moisture variation was reduced to a minimum, although it was found impossible to maintain exactly comparable moisture conditions at various soil temperatures. Since the critical range of soil moisture had been previously determined, it seemed logical to believe that the two factors,

moisture and temperature, could be separated and their individual influence studied.

In the field the temperature and moisture experiments were handled by sowing at different dates during the fall and spring. Continuous soil-and-air temperature records were secured by means of the soil-air thermograph, which was checked each day by electrical resistance thermocouples



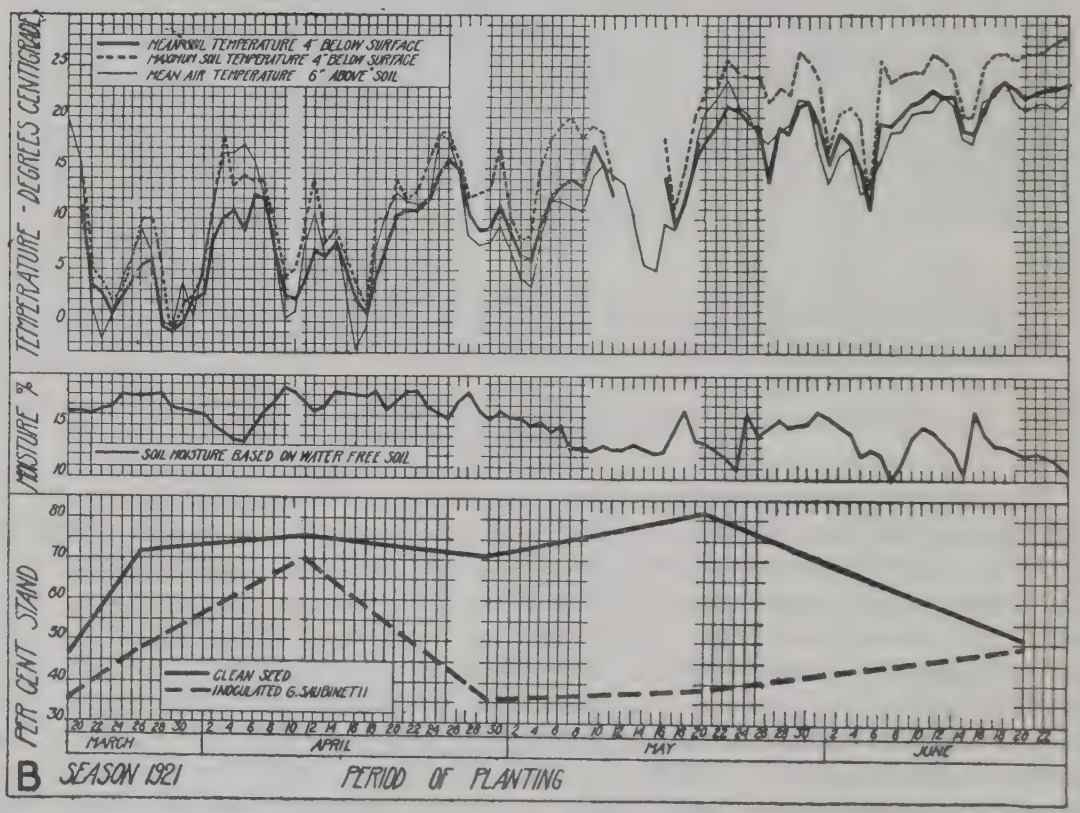


FIG. 2.—Graphs showing soil and air temperature for the season of 1921 and percentage of stand of spring wheat and corn sown on different dates. A, Corn; B, Marquis wheat. The squares of the graph paper are filled in for the seedling period of each sowing, as, for instance, August 24 to 29. This represents the period during which the environmental factors influenced the development of seedling-blight. To avoid confusion the curves for percentage of stand of plants are plotted on the date sown. The distance between the solid lines and broken lines showing stand of check and inoculated plots represents the percentage of seedling-blight.

placed immediately adjacent to the soil-thermograph bulb and left undisturbed for the duration of the experiment. The instrument bulbs were placed in the plots used for the experimental studies at depths of 2 and 4 inches. Sowings were usually made after a rainy period when the moisture content of the soil was at about 15 per cent, based on water-free soil (fig. 2). In some few cases it was necessary to increase the soil-moisture

content by sprinkling the plot the day before planting. Soil-moisture determinations were run before each seeding to insure similar moisture conditions. Thus temperature was the chief soil variant for each seeding, at least during the seedling stage. Of course light intensity and the duration of the light varied for the different seedings, and such factors must be taken into consideration in all work of this type.

INOCULATION TECHNIC

In both wheat and corn the inoculations were made by using a water suspension of conidia of *Gibberella saubinetii*. The conidia were obtained from 3-to-4-day-old cultures. Comparative inoculations with these vegetative conidia and the conidia produced at the end of the vegetative period gave the same type and percentage of blighting. Therefore the vegetative conidia were used, as they could be produced much more rapidly and uniformly.

Sufficient seed for each can of the series was soaked for 10 minutes in the water suspension of the conidia, after which each kernel was lifted out separately with sterile forceps and placed in the sterilized soil in the can. The controls were similarly treated in sterile distilled water.

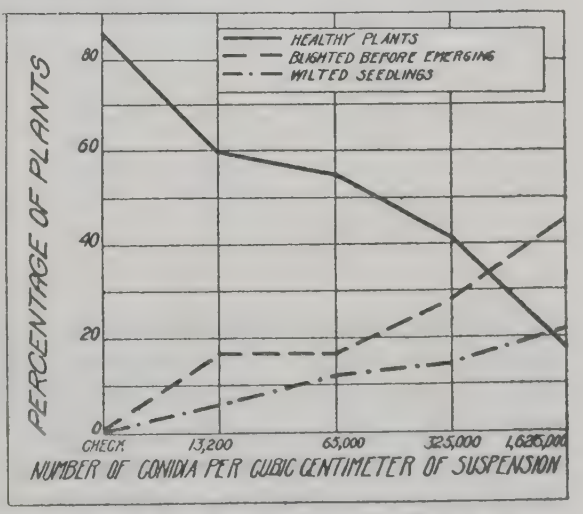


FIG. 3.—Graph showing relation of number of conidia in the inoculum to the development of seedling-blight of wheat. The increased rate of application of conidia in the inoculum on the seed gradually increased both the severity of the blighting and the percentage of plants blighted before emerging.

In several of the early inoculation experiments the number of conidia in the suspension seemed to have a marked influence not only on the percentage of blight produced but also upon the type of blight that resulted. At the outset, therefore, it was necessary to take up a study of this phase of the problem and determine the influence of the number of conidia introduced with the seed on the percentage of blight occurring and the type of blight developing, as well as to determine what spore concentration was most capable of producing a consistent and uniform type of blighting.

The regular temperature cans were prepared, as previously described under the discussion of methods, and filled with soil at 60 per cent of the moisture-holding capacity. The cans were placed in the greenhouse at a temperature of 16° to 20° C. Uniformly plump seed of both Marquis and Turkey wheat was soaked in conidial suspensions ranging from about 13,200 conidia per cubic centimeter to 1,625,000 conidia per cubic centimeter in the most concentrated spore suspension used. These suspensions were prepared by making up a large quantity of a heavy suspension of conidia obtained from vigorous young cultures. The average of a large number of conidial counts made on the haemocytometer was taken as the basis for making the spore dilutions.

With the increase in the number of conidia, there resulted a marked increase in the percentage of diseased plants. Further, with the increase

in the concentration of inoculum, there were gradual increases in both the percentage of seedlings killed preceding emergence and the percentage of those wilted following emergence (Table I and fig. 3). In the more dilute suspensions the attack was usually restricted to the subcrown internode [4] and resulted in a slight blighting, from which in many cases the seedlings recovered. As the number of conidia increased, however, the attack became more general, often including the young plumule, thus resulting in the higher percentages of seedlings which blighted before emergence. A combination of a heavy conidial suspension, together with conditions favorable for the development of the blight, usually resulted in high percentages of the seedlings being killed before emerging (Pl. 5, A).

TABLE I.—*Relation of number of conidia to the production of seedling-blight of wheat and to the type of blight produced*

Average number conidia per cubic centimeter of inoculum.	Percentage germination of wheat.	Percentage of plants emerged.	Percentage of disease-free plants.	Percentage of blighted plants.		
				Slightly diseased.	Wilted.	Blighted before emerging.
Control	99	98	85	13	0	1
13,200	97	80	60	13	7	17
65,000	99	78	56	9	13	21
325,000	93	65	43	7	15	28
1,625,000	90	45	18	6	21	45

The two intermediate spore dilutions seemed to give the most uniform results. The first, 65,000 conidia per cubic centimeter, gave a medium percentage of both wilt and blight, whereas the second dilution, 325,000 conidia per cubic centimeter, gave a relatively higher percentage of seedlings blighted before emergence. Consequently these two spore dilutions were used for inoculation purposes in the soil-temperature and soil-moisture experiments reported in this paper.

TEMPERATURE AND MOISTURE EXPERIMENTS IN THE GREENHOUSE

INFLUENCE OF TEMPERATURE ON THE PARASITE [5]

A considerable literature has been accumulated on the influence of temperature, moisture, and other environing conditions on the development of both saprophytic and parasitic species of the genus Fusarium. Schnaffnit (*25*), Humphrey (*18*), Gilman (*11*), Tisdale (*30*), and others have found that, in general, these organisms develop best at comparatively high temperatures. Their results, however, show that while this group of fungi have fairly high optima, yet they have a very wide temperature range, spore germination, vegetative development, and spore production taking place at a range of from 5° to 36° C.

[4] The designation "subcrown internode," as used for wheat in this paper, applies to the structure between the base of the coleoptile and the crown node.

[5] From unpublished data of experiments conducted by Helen Johann, Grace O. Wineland, and the writer in the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

SPORE GERMINATION

Conidia from several different strains of *Gibberella saubinetii* were germinated in the Altmann incubator and temperature chambers referred to under methods, at temperatures ranging from 4° to 36° C., in hanging drop cultures of distilled water, soil decoction, and potato-dextrose agar, as well as on poured plates of potato-dextrose agar, and on young wheat seedlings growing in sterile, moist soil. The influence of temperature on rate and percentage of spore germination is shown for one medium, distilled water, in Table II.

TABLE II.—*Influence of temperature on the germination of conidia of Gibberella saubinetii in hanging drops of distilled water*

Mean temperature.	Average germination and length of germ tube.							
	6 hours.		24 hours.		48 hours.		96 hours.	
	Germination.	Length.	Germination.	Length.	Germination.	Length.	Germination.	Length.
°C.	*Per cent*	μ	*Per cent.*	μ	*Per cent.*	μ	*Per cent.*	μ
3.6	0	0	0	0	0.5	0	88	45
11.0	0	0	80	75	86.5	100	.....	.....
17.0	94	100	.....	.....	.....	.....	.....	.....
28.5	97	100	.....	.....	.....	.....	.....	.....
35.0	84	40	.....	.....	.....	.....	.....	.....

The results show that germination and growth take place over a wide temperature range. The cardinal temperatures for germination and growth of the germ tubes were not greatly altered by the use of other media than those given in Table II. The inhibiting influence of the extreme temperatures, however, was more marked in unacidified media. The optimum temperature for both rate and percentage of germination was raised about 4°C. by acidifying the potato-dextrose agar with one drop of 25 per cent lactic acid in each tube of agar. Good germination was obtained from 4° to 32°, but above this temperature the conidia became very much swollen, with much thickened walls and twisted, abnormal germ tubes.

The temperature range for ascospore germination was found to be essentially the same as that for the conidia.

VEGETATIVE DEVELOPMENT

Potato-dextrose agar, both acidified and unacidified, was poured into Petri dishes, care being taken to have dishes of the same size and the same quantity of agar in each plate. A dilute conidial suspension was prepared, and a single loopful was placed in the center of each plate for the series started from conidia. Disks of equal size were cut from the outer circumference of a young colony on agar and placed in the center of the plates for the series started from mycelium. These plates were then incubated in a saturated atmosphere in the temperature chambers previously described under methods, at temperatures ranging from 8° to 36°C. and in the Altman incubator at temperatures ranging from 4° to 30°. The rate of growth was determined by measuring the diameter

of the resulting colonies from day to day. The best comparative data were obtained when the cultures were 4 to 6 days old.

A sparse, slow growth occurred at 3° to 4° C., a light growth developed at 5°, and an appreciable amount of mycelium developed at 8° after an interval of 10 to 12 days. Above this temperature, the rate of growth increased rapidly, the optimum falling at 24° with unacidified agar, and at 28° with acidified agar. Little or no growth occurred above 32° to 34°, regardless of the time incubated. The average diameters of the colonies are given in Table III and shown graphically in figure 4. The diameter and texture of the colonies of a single series are shown in Plate 3, C.

TABLE III.—*Influence of temperature on the vegetative development of Gibberella saubinetii incubated on potato-dextrose agar*

Reaction of medium and inoculum.	Average number determined.	Age of culture (days).	Average diameter of colonies in centimeters at different temperatures.							
			8° C.	12° C.	16° C.	20° C.	24° C.	28° C.	32° C.	36° C.
Unacidified agar, conidia.	8	4	0.1	1.3	2.5	5.9	8.1	7.5	0.3	0
Acidified agar, conidia..	8	4	0	.7	1.7	3.0	3.1	5.7	.1	0
Unacidified agar, mycelium.....	8	4	1.5	2.5	4.5	6.5	9.0	7.3	1.6	.1
Acidified agar, mycelium.	9	4	.7	1.4	2.3	2.7	4.5	6.0	1.3	0

The cardinal temperatures for mycelial growth were about the same when the fungus was incubated on the stems of young wheat seedlings growing in tubes of sterile soil, the optimum rate of growth ranging from 24° to 28° C. No mycelium was found on the seedlings at temperatures above 32° to 34° C.

PRODUCTION OF CONIDIA

Observations on the production of conidia by the fungus were made on potato-dextrose agar poured plates, in hanging drops of potato-dextrose agar, and on the stems of young wheat seedlings growing in tubes of sterile soil. Conidia were produced at temperatures ranging from 4° to 34° C., as shown in Table IV.

TABLE IV.—*Influence of temperature on the production of conidia by Gibberella saubinetii in hanging drops of potato-dextrose agar*

Time incubated.	Rate of production and comparative number of conidia produced at different temperatures.							
	4° C.	11° C.	17° C.	24° C.	28° C.	30° C.	32° C.	34° C.
24 hours.....	0	0	0	1	a 3g	10g	10g	0
2 days.....	0	0	3	10g	10g	10g	10g	2
3 days.....	0	0	10g	10g	10g	10g	10g	3
4 days.....	0	1	10g	10g	10g	10g	10g	4
6 days.....	0	10	10g	10g	10g	10g	10g	4
9 days.....	0	10g	.....	.....	.....	.....	.....	.....
18 days.....	0	.....	.....	.....	.....	.....	.....	.....

[a] Letter g following a number indicates new conidia germinated after being produced.

The time required for the production of conidia varied from about 20 days at 4° C. to 24 hours at 30° to 32°. Sporodocia-like tufts of conidia were produced at the higher temperatures almost as an outgrowth of the conidium. The higher temperatures stimulate the early production of conidia by *Gibberella saubinetii*, as described by Dickson and Johann (9).

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF HOSTS

While it is not the purpose of this paper to discuss in detail the influence of temperature on the development of wheat and corn, this being reserved for another paper, yet it is desired to give a brief summary in order to be able to present more clearly the relation of the hosts to the development of the disease. The results obtained have demonstrated that temperature is one of the major environmental factors in the physiology of the cereal crops; and, therefore, a knowledge of these temperature responses is of fundamental importance in explaining predisposition to disease. The experiments likewise have shown that other factors, such as soil moisture and light intensity, greatly influence predisposition to disease. Either of these factors, for instance, under certain conditions, may completely overshadow temperature. The study of environmental factors concerned in the development of the seedling-blight has served to bring out clearly the fact that all the environing conditions are inseparably connected and that the degree of blight is the product of a number of factors, any one of which when varied may start a similar set of reactions within the host or parasite. It seems necessary, therefore, to study the responses set up by these environing factors, not only in the parasite but in the healthy host as well.

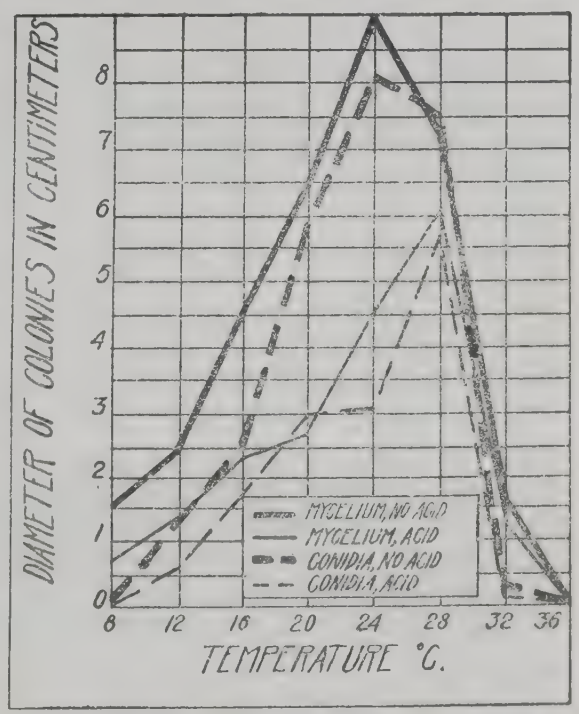


FIG. 4.—Graph showing relation of temperature to the vegetative development of *Gibberella saubinetii* in artificial culture. The optimum temperature for growth, based on maximum diameter of colony, was 24° C. on unacidified agar and 28° on agar acidified with one drop of 25 per cent lactic acid per Petri-dish plate.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF MARQUIS WHEAT

Uniform, plump kernels of Marquis wheat were selected for control and inoculated series alike, treated with mercuric-chlorid solution and sown in the soil temperature cans. The soil had been adjusted to the proper moisture content and placed in the cans several days previously. The wheat kernels were planted at a uniform depth of 1.5 inches, after which the cans were covered for 36 hours to prevent excessive evaporation from the soil surface. After uncovering and mulching, the containers were made up to their original weight each day throughout the duration of the

experiments by adding water at the same temperature as the soil. Data were taken on the number of plants emerging, rate of emergence, general appearance, rate of tillering, number of tillers; and in some few instances the plants were grown to maturity and the influence of soil temperature on maturation studied. Upon completion of the experiments the roots were carefully washed free from the soil, after which the length of roots, length of the subcrown internode, height of the plants, and dry weight of roots and tops were determined separately. The data discussed in this chapter are given in condensed form in the seedling-blight discussion. The data indicate that the influence of soil temperature on the cereals tested seems to be greater during the germination period than at any other stage of their development. This is not, however, a new observation, as Gutzeit (*12*) and others have shown this to be true for other plants than the cereals. Sachs (*24*), Haberlandt (*13; 14, p. 39–47, 69–78*), Bialoblocki (*5*), Vinall and Reed (*31*), and others have determined the cardinal temperatures for the germination of wheat, corn, and other cereals. Still others, notably Kincer (*22*), have attempted to correlate the critical temperatures for germination and date of seeding.

The spring wheat emerged first at about 28° C., at which temperature it reached the surface in 38 to 48 hours. The rate of emergence decreased rapidly above this soil temperature—28°—and rather gradually below that temperature. The seedlings produced at soil temperatures above 28° emerged very irregularly and developed unevenly. On the other hand, growth was very uniform at the lower soil temperatures, even at 8°. At this temperature it required 10 to 12 days for the plants to emerge.

The comparative rate of development of roots and plumules of the wheat seedlings at low and high soil temperatures was indicative of a distinctly different type of metabolism at the two temperature extremes. At the low temperatures, notably 8° and 12° C., the roots developed first and usually were several centimeters long before the plumule began to develop; whereas at the high temperatures, notably 28° to 32°, the plumule was well out of the soil before the roots developed. The low temperatures continued to stimulate root development until well towards the heading period, the largest root system for spring wheat developing at about 12° to 16°.

Elongation of the subcrown internode, the first internodal region between the seed and the first node, was greatly stimulated by high soil temperatures. At 24° C. and above, the secondary roots developed at or near the soil surface, while at 8° subcrown internode elongation was almost entirely inhibited, the first node and secondary roots developing immediately above the seed. This inhibition of the subcrown internode at low temperatures persisted even when the seeds were sown at considerable depths below the surface. At temperatures of 16° or above, however, deep seeding or a moist dark compartment greatly increased the length of this region, bringing the secondary roots near the surface of the soil. The response of these tissues to environing factors has been described by Schroeder (*26*), Vogt (*32*), Halsted and Waksman (*15*), and others.

Elongation of the coleoptile, on the other hand, was inhibited by high soil temperatures. At temperatures above 20° C., the growing point of the culm always broke through the coleoptile before emerging from the soil. At temperatures below 16°, however, the coleoptile elongated very much faster than the growing point of the culm and as a result inclosed the growing culm until after emergence, thus preventing the young

growing tip from contact with the soil or soil-inhabiting organisms. At 8°, for instance, the coleoptile usually was 2 to 4 cm. above the surface of the soil before the first leaf pushed through. This variation in type of emergence may help explain the protective influences of low soil temperatures against certain diseases of the small grains.

The largest top production during the early seedling stage was at 20° to 24° C. As the plant increased in age there was a gradual lowering of the optimum temperature for development of tops, until in the later stages of development the best top development resulted at 12° to 16°.

The rate and extent of tillering of spring wheat was modified at the extremes of soil temperature, both high and low. The plants tillered first at the temperatures producing most rapid emergence, namely 24° to 28° C., which indicated somewhat similar cardinal temperatures at this stage of development. Only a limited number of tillers formed at 32° and above. Below this, soil temperature had little effect on tillering until at 8°, where the tillering was limited to two or three culms per plant. The maturation of the wheat plant was hastened by low soil temperatures. Hutcheson and Quantz (*19*) and Appel and Gassner (*2*) have shown this to be true for several of the small grains. The plants grown continuously at a soil temperature of 16° were the first to head and mature. The time of maturation increased slightly with variation in each direction, the plants at 8° maturing at approximately the same time as those at 24°. At temperatures of 24° and above, the heading was retarded very considerably, internodal elongation was reduced, and leaf production stimulated, the spring-wheat plants at 28° heading when only 10 to 12 inches high. Heading was almost entirely inhibited at 32° and above, regardless of the season of the year.

While spring wheat germinated more rapidly at soil temperatures of 24° to 28° C., the germination was more uniform and stronger plants resulted at the lower temperatures—about 8° to 16°. The greatest development of roots as well as of tops occurred at the lower soil temperatures. The earliest maturing, most stocky, and best filled plants resulted at soil temperatures of about 16°.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF TURKEY WHEAT

The cardinal temperatures for the development of Turkey winter wheat were, in general, about 4° C. below those for spring wheat. The responses were very similar, except that winter wheat tillered excessively at soil temperatures of 24° and above, and maturation was inhibited by this and higher soil temperatures. Plants grown continuously at soil temperatures of 28° and above were not only checked in their internodal elongation, but failed to form even the embryonic floral organs, the leafy vegetative development continuing throughout the season. Chemical analyses showed this to be a carbohydrate nitrogen balance similar to that described by Kraus and Kraybill (*23*) in the tomato. The analytical data will be discussed in detail in a later paper.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF CORN

Disease-free, uniformly matured corn was selected for all the temperature experiments. Usually the seed for the entire series of experiments was taken from a single ear. The corn was planted 1.5 inches

deep after the cans had been prepared as previously described for the wheat series.

Corn emerged first at the soil temperature of 32° C. The rate of emergence decreased gradually below this temperature to 24°. Below this it decreased relatively rapidly to 12°, where emergence occurred in about 14 to 16 days. These results correspond very well with those given for corn by Sachs (*24*), Haberlandt (*13; 14, p. 39–47, 69–78*), Halstead and Waksman (*15*), Vinall and Reed (*31*), and others. At a soil temperature of 8°, although germination occurred, the growth processes were slowed up to such an extent that the plumule rarely reached the surface. At the end of two or three months' time, at this low temperature, the roots elongated 10 to 12 cm. and the plumule had grown several centimeters when the reserves were exhausted and the plant succumbed to the attack of various soil organisms. Only occasionally at 8° did a few plants emerge and become green. The uniformity of emergence and stand decreased rapidly below 20°.

The seedlings produced at the low soil temperatures were stocky, very succulent, and brittle. The leaves were dark green at soil temperatures from 24° to 16° C. Below this the intensity of green decreased until at 12° the leaves were distinctly chlorotic.

The influence of soil temperature on the comparative development of the root and plumule of the corn seedling was similar to that in the wheat seedling, although not so marked. Root development was stimulated at the lower soil temperatures and plumule development inhibited, especially the growing tip, the coleoptile elongating much more rapidly than the shoot. As in wheat, the first leaf did not rupture the coleoptile at the low temperatures until entirely out of the ground.

The largest root system, irrespective of the age of the plant, developed at soil temperatures of about 20° C. The heaviest, as well as the longest, tops developed at about 24° during the earlier period of growth and at about 28° in the later stages of development.

Corn, unlike wheat, matured faster at the higher temperatures, the floral parts developing first at about 32° to 28° C. It has not been possible, due to the retarded vegetative development, to mature corn in the tanks at soil temperatures below 16°.

In general, then, corn functioned at much higher soil temperatures than wheat, the optimum for emergence was 32° C., and the largest, most uniform seedlings resulted at 24° to 28°.

These results indicate that seedlings of spring and winter wheat and corn develop most vigorously and most uniformly at the following soil temperatures: Spring wheat, 16° to 20° C.; winter wheat, 12° to 16°; and corn 24° to 28°.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF SEEDLING-BLIGHT IN MARQUIS WHEAT

Studies on the influence of temperature on the development of the parasite have shown that germination of the conidia and vegetative growth take place over a wide temperature range from below 8° to about 32° C. Accordingly a set of experiments was started to determine the range and optimum temperature for infection of wheat seedlings.

Uniform disease-free kernels were treated with mercuric chlorid and after washing thoroughly were soaked in a water suspension of conidia of *Gibberella saubinetii* for 10 minutes, lifted out with sterile forceps,

and placed in the sterilized soil in the cans. All series except two were inoculated with a spore suspension of 65,000 conidia per cubic centimeter. Two series were given a heavier inoculation with 325,000 conidia per cubic centimeter. General growth data were taken on the inoculated and control plants of each series. In addition, daily notes were taken on the number of seedlings showing slight blighting and wilting, and finally upon the completion of the experiments the soil was carefully washed on a screen and each kernel examined for evidence of germination. The columns in Tables V to VII headed "average percentage of germination" represent the total number of kernels which had germinated. The percentage of kernels germinating, the percentage emerging, the percentage of disease-free seedlings, and the percentage of blighted kernels were recorded for each series. The data presented in Table V represent an average of eight different experiments. The results from each were uniform enough to warrant taking an average and explaining the few variations in the discussion of results.

TABLE V.—*Influence of soil temperature on the development of seedling-blight in Marquis wheat*

[Average of eight experiments.]

Average soil temperature.	Average number of days to emerge.	Control.		Inoculated with *Gibberella saubinetii*.		
		Average of percentage germination.	Average percentage of healthy plants.	Average percentage of germination.	Average percentage of healthy plants.	Average percentage of blighted plants.
°C.						
8	16	80	80	84	84	0
12	10	80	80	78	61	17
16	7	79	77	70	48	22
20	7	81	79	75	41	34
24	6	75	71	68	49	19
28	4	74	68	65	43	22
32	4	77	71	73	54	19
36	6	41	16	51	38	13

The influence of soil temperature on the parasite and the hosts has been discussed in detail earlier in the paper. The general rate of development of the seedling, that is, the rate of emergence and rate of tillering and maturing, was not greatly altered by the presence of the parasite. The number of plants emerging, the general appearance of the plants, the length of roots, and the height of plants as well as the weight of both roots and tops, however, were greatly modified by the presence of the parasite.

The germination of spring wheat inoculated with conidia of *Gibberella saubinetii* was reduced by soil temperatures between 12° and 28° C. At a soil temperature of 8°, however, the germination was equal to that of the controls in every series grown. At 12° the germination was reduced, on the average, only 2 per cent (Table V and fig. 5), while at soil temperatures of 16° to 28° the germination was lowered about 8 per cent in each case. The growth of the parasite apparently was sufficiently rapid to penetrate and kill a number of the embryos before they had increased greatly in size, thus reducing germination very

materially within this temperature range. At 32° and above the reduction in germination of the inoculated series was less, in many of the series the percentage germination being as high as that in the controls.

At these higher temperatures, 32° and 36° C., the germination of both controls and inoculated seed was reduced by what was apparently Trichoderma, Penicillium, and other soil organisms attacking the endosperm. The germination of the wheat or corn was not indicative of the extent of damage done by the organism, since many of the kernels germinated and were blighted afterward.

The emergence of the seedlings, therefore, was a better indication of early blighting, although in many cases, especially at soil temperatures of 16° and 20° C., 25 per cent of the seedlings blighted after emerging. The final index, therefore, was the percentage of disease-free and blighted plants at the close of the seedling stage, or about the fourth-leaf stage. Ater this period little, if any, blighting occurred.

The seedling-blight of spring wheat did not occur at soil temperatures of 8° C. or below unless moisture and other environing factors were varied outside of their critical range. At 12°, which was about the critical soil temperature for the production of seedling-blight, both in the tanks and in the field, series grown with a low light intensity blighted badly, whereas series grown in good light showed only a relatively medium degree of blight. In two series of Marquis wheat the temperatures were set at 10° instead of 12°, with the result that seedling-blight was reduced 75 per cent below that at a soil temperature of 12°. The blight was most severe at soil temperatures of 16° to 24°. At this range the seedlings were blighted before emerging and also showed yellowing and wilting during the first-leaf and second-leaf stages. At the higher temperatures, 28° and higher, the blighting usually occurred after the seedlings had emerged. At these temperatures also, as previously stated, the reduction in germination by the parasite became less marked. The maximum rate of emergence of the seedling occurred at about this same temperature range. In all probability, therefore, the seedling developed so rapidly at these temperatures that infection did not take place until after emergence (Pl. 5, C.).

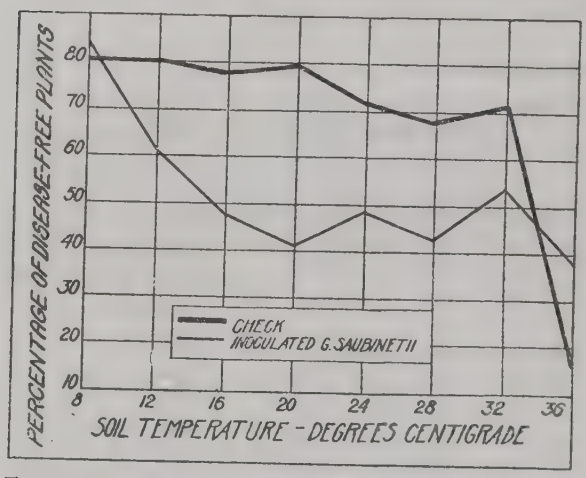


FIG. 5.—Graph showing relation of soil temperature to the development of seedling-blight of Marquis wheat. Average of eight experiments. The distance between the heavy and light lines shows the percentage of seedling-blight. The wheat seedlings blighted at temperatures from about 12° to about 34° C.

The average dry weight per plant, of roots and of tops, was the same in the inoculated and uninoculated series at the soil temperature of 8° C. At soil temperatures of 16° to 28° the weight of both roots and tops in the inoculated series decreased several per cent below that in the controls, which indicated the action of the parasite on the roots of the remaining plants even though blighting did not occur.

At the higher temperatures, 32° to 36° C., there was considerable reduction in the germination and some blighting in both control and inoculated series, especially when grown in nondisinfected soil. Isolations from such seedlings gave some *Gibberella saubinetii* at 32° but

none at 36°. The preponderance of platings gave what was apparently Trichoderma, Penicillium, Rhizopus, and Aspergillus in the order named. These organisms generally invaded the endosperm and developed from there into the seedling.

At other soil temperatures, 12° to 28° C., *Gibberella saubinetii* was universally reisolated, generally in pure culture even in unsterilized soil, if the seedlings were plated before they were badly decomposed. At soil temperatures of 20° to 28° perithecia were often formed in gregarious masses on the first-leaf sheath of the blighted seedlings near the surface of the soil.

Infection takes place and the blight progresses, then, at or above 12° C., but it is rather evident that, for very severe development of the blight, a soil temperature above 16° and below 28° is necessary. Since the attack of the organism is chiefly on the young seedling, the range of temperature during the "nursing period" is undoubtedly the important factor in determining the severity of this stage of the disease.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF SEEDLING-BLIGHT IN TURKEY WHEAT

The seven different series of Turkey wheat were handled as previously described for Marquis wheat experiments, except that all of the winter wheat was grown in the fall or early winter. All the series were inoculated with the more dilute conidial suspension, containing 65,000 conidia per cubic centimeter of solution. Data similar to those obtained for Marquis wheat were obtained in all cases. The average results of the seven experiments are given in Table VI and shown graphically in Figure 6.

TABLE VI.—*Influence of soil temperature on the development of seedling-blight in Turkey wheat*

[Average of seven experiments.]

Average soil temperature.	Average number of days to emerge.	Control.		Inoculated with *Gibberella saubinetii*.		
		Average percentage of germination.	Average percentage of healthy plants.	Average percentage of germination.	Average percentage of healthy plants.	Average percentage of blighted plants.
°C.						
8	18	98.0	97.0	96.0	96.0	0
12	12	89.4	89.4	73.1	67.1	6.0
16	8	95.5	95.5	60.1	47.3	12.8
20	6	96.5	93.0	61.7	44.7	17.0
24	5	82.5	82.5	67.6	49.3	18.3
28	4	80.4	77.7	56.1	35.0	21.1
31	6	62.5	62.5	59.2	57.1	2.1
35	10	48.0	43.7	25.9	25.9	0

The response of winter wheat to the blight was similar, in almost all cases, with that described for spring wheat in the previous pages. The only differences noticeable were that blighting was more severe at 20° in the Marquis series and was less extensive in the winter wheat seedlings at a soil temperature of 12° C. The average percentage of blighted seedlings in the winter wheat was less at all temperatures, but

as all of these series were inoculated with the suspension of 325,000 conidia per cubic centimeter, these differences can be partially explained. There was, however, some evidence that Turkey wheat was less susceptible to the blight than the Marquis wheat. More experiments are necessary with these two wheat varieties to confirm these results.

In comparing the influence of temperature on the parasite in pure culture and on the production of the blight of wheat seedlings, the temperature influence at first thought seems rather simple. It appears that wheat is capable of growing at soil temperatures lower than those at which the parasite can develop and become parasitic. The wheat plant, therefore, escapes the disease, the blight occurring only at temperatures which stimulate the growth of the parasite. Such a relationship was pointed out by Clayton[6] in the tomato-wilt caused by *Fusarium lycopersici* Sacc., where the wilt curve produced in response to soil temperature coincides with the growth curve of the organism in pure culture, the optimum for both occurring at about 28° C. Upon closer analysis, however, the relationship seems far more complex and distinctly different from that existing in the tomato-wilt problem.

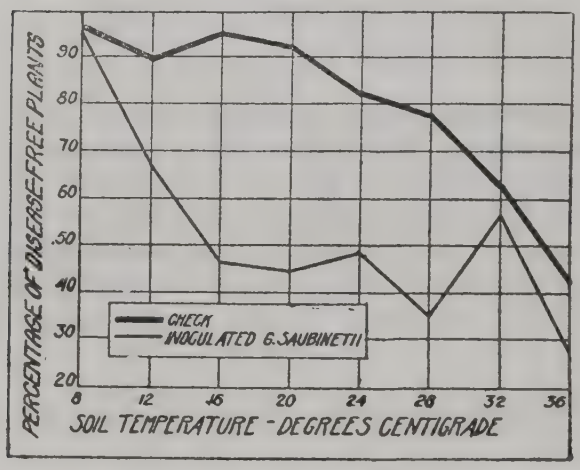


FIG. 6.—Graph showing relation of soil temperature to the development of seedling-blight of Turkey wheat. Average of seven experiments. The distance between the heavy and light lines shows the percentage in seedling-blight. Compare with spring wheat of figure 5.

The parasite has an extremely wide temperature range for both spore germination and growth, and furthermore, while a histological study of the wheat seedlings at the low temperatures demonstrated an abundance of the mycelium of the parasite around the subterranean portions of the seedlings, yet no penetration could be detected. The hyphae were twisted around the root hairs and matted on the cortex of the roots and coleoptile. In many places swollen tips of the hyphae were pressed tightly into the intercellular spaces on the epidermis of the coleoptile and against the root hairs, yet were seemingly unable to penetrate between the cells or gain entrance through the membrane of the root hairs, whereas at the high temperatures the hyphae readily penetrated any of the young seedling tissues, gaining entrance through the root hairs as well as through the epidermal cells of the coleoptile, subcrown internode, and roots.

These studies, then, seemed to indicate a relationship at least between host and parasite almost the reverse of that in the tomato-wilt. The influence of environment upon the host appeared to be the fundamental cause of the response. The wheat seedling seemingly was unable to react favorably to the higher soil temperatures and thus succumbed to the blight. The results have shown that a short exposure to high temperatures during the germination period unbalanced the wheat seedling and thus made it susceptible to the parasite.

[6] From unpublished data of studies conducted in the Department of Plant Pathology, University of Wisconsin.

INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF SEEDLING-BLIGHT IN CORN

The experiments on the relation of soil temperature to the seedling-blight of wheat have shown that blight does not occur much below 12°C., and that at 12° and above the wheat seedling becomes susceptible to attack. The investigations seem to demonstrate further that the response of the host to soil temperature rather than that of the parasite must be considered as the chief explanation of these differences. Accordingly, another series of experiments was started with a different host, namely, corn, which is a comparatively warm-climate crop and is susceptible to the same parasite, *Gibberella saubinetii*.

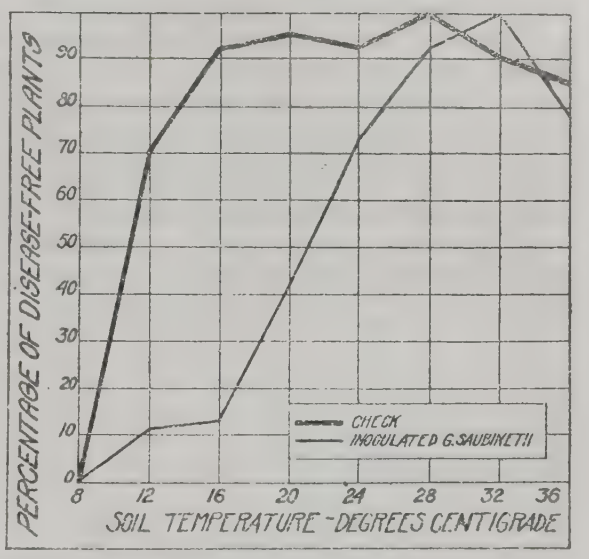


FIG. 7.—Graph showing relation of soil temperature to the development of seedling-blight of corn. The distance between the heavy and light lines shows the percentage of seedling-blight. Compare with seedling-blight of wheat in figures 5 and 6.

The seed for each series of experiments was taken from the same ear of corn. The method of inoculation and planting was identical with that previously described for wheat. Observations comparable to those recorded for wheat were made; in fact, in all but two series wheat was grown simultaneously with corn in the same temperature tanks. The data given in Table VII and shown graphically in figure 7 represent the average of seven different series of corn experiments.

TABLE VII.—*Influence of soil temperature on the development of seedling-blight of corn*

[Average of seven experiments.]

Average soil temperature °C.	Average number of days to emerge.	Control.		Inoculated with *Gibberella saubinetii*		
		Average of percentage germination.	Average percentage of healthy plants.	Average percentage of germination.	Average percentage of healthy plants.	Average percentage of blighted or diseased plants.
8	.....	50	.....	0	0	0
12	15.7	77	72	20	11	9
16	9.7	96	92	54	12	42
20	5.7	97	95	89	42	47
24	5.0	92	92	100	73	27
28	3.6	100	100	94	93	1
32	3.0	97	90	100	100	.....
36	3.6	98	85	97	77	[a] 20

[a] No blighting; killed chiefly by high soil temperatures.

The rate of development, at least during the seedling stage, was checked slightly by the introduction of the parasite at soil temperatures below 24° C., but above this no differences were detected.

The germination of the corn inoculated with conidia of *Gibberella saubinetii* was influenced by soil temperatures between 8° and 20° C. At a soil temperature of 8° the embryos of all the kernels were invaded and killed before germination. Germination was reduced an average of 57 per cent below the controls at 12° and 42 per cent at 16°. At 20° the reduction in germination was strikingly less, only 8 per cent, and at 24° all the kernels germinated in the seven series grown.

Only a few of the seedlings emerged in the controls and none in the inoculated series at 8° C. At 12° about 50 per cent of the seedlings in the inoculated series reached the surface before blighting, the remaining plants usually blighting when the plumule was from 1 to 3 cm. long. Above this temperature practically all the seedlings emerged and wilted while in the first-leaf or second-leaf stage (Pl. 4).

All the corn plants at 8° C. blighted; all but about 12 per cent blighted at 12°; about half of them blighted after wilting at 20°; only a few wilted at 24°, and these were chiefly in two series of a very suscept-

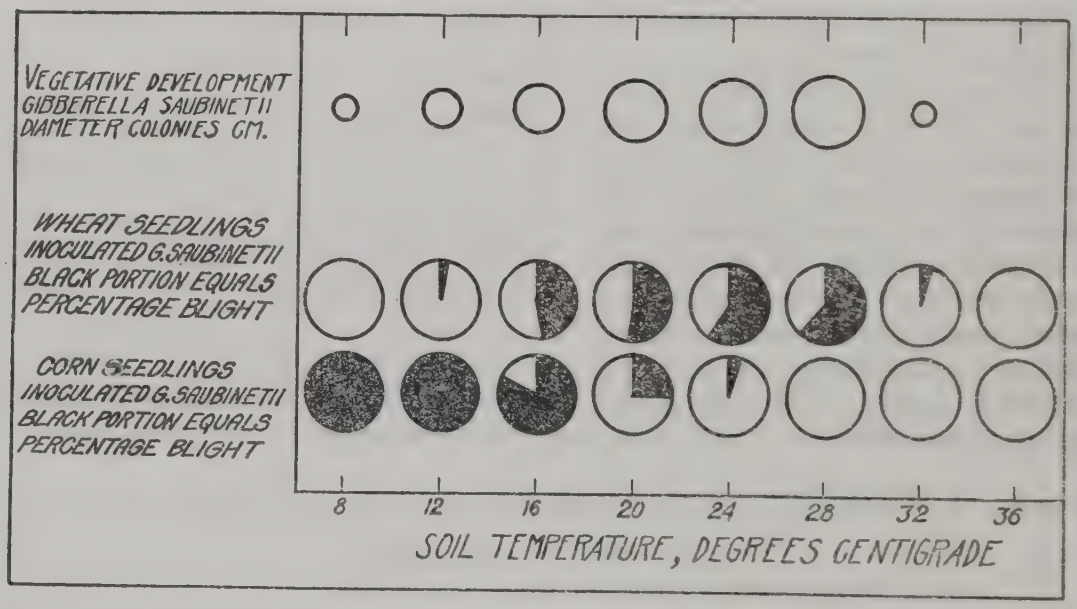


FIG. 8.—Graph showing relation of soil temperature to the vegetative development of the parasite and the production of the disease. A graphic summary of the vegetative development of *Gibberella saubinetii* in artificial culture and the comparative quantities of seedling-blight of corn and wheat at different soil temperatures. The black portion of the circle shows the average percentage of blighted seedlings.

ible strain of Ninety-Day corn. Many of the plants showed root lesions and some were weakened by the root attack but usually were capable of recovering and later making a medium growth, especially if put at the higher temperatures. A few plants at 28°, in the two series previously mentioned, showed small root lesions but none sufficient to hinder the development of the plant. The blight was most severe at 8° to 16°, where only a few plants survived, and stopped abruptly at 24°. At this temperature root lesions occurred in a few cases but in most of the series did little damage to the plants.

The average dry weight per plant of roots and tops of the so-called disease-free plants in the inoculated series at temperatures from 12° to 24° C., was much below that of the control plants; above this temperature the weights were approximately the same.

The parasite was isolated in pure culture from the seedlings, the endosperms, and the roots of the blighted and diseased seedlings. After remaining in the soil a week or two there was a development of sapro-

phytes, especially bacteria, which often crowded out *Gibberella saubinetii* entirely.

In corn, infection takes place and blight progresses, then, at all temperatures below 24° C. The most rapid development of the disease and the maximum blighting occur at 12° and 16°. Below this, namely, at 8°, where wheat is entirely resistant to the attack of this parasite, corn succumbs entirely (fig. 8 and Pl. 4 and 5).

As the temperature range for the production of blight in this warm-climate plant is the reverse of that for wheat, a cool-temperature plant, it is only logical to say that this parasite is capable of attacking both hosts during their seedling stages when they are subjected to unfavorable environmental conditions. This fact is brought out even more strikingly in the following combined moisture and temperature studies.

INFLUENCE OF SOIL MOISTURE ON THE DEVELOPMENT OF SEEDLING-BLIGHT IN WHEAT

Earlier studies conducted in connection with this problem demonstrated that the influence of moisture on the seedling-blight of wheat was similar to that of temperature,[7] for low soil moistures increased the susceptibility of the wheat seedling and thus increased the virulence of the attack. The influence of soil moisture gradually decreased until at 45 to 50 per cent of the moisture-holding capacity of the soil this factor ceased to influence blighting. As a consequence all the temperature experiments were conducted with a soil at 60 per cent of the moisture-holding capacity, assuming that in this way moisture could be separated from temperature and the critical range of the latter be determined.

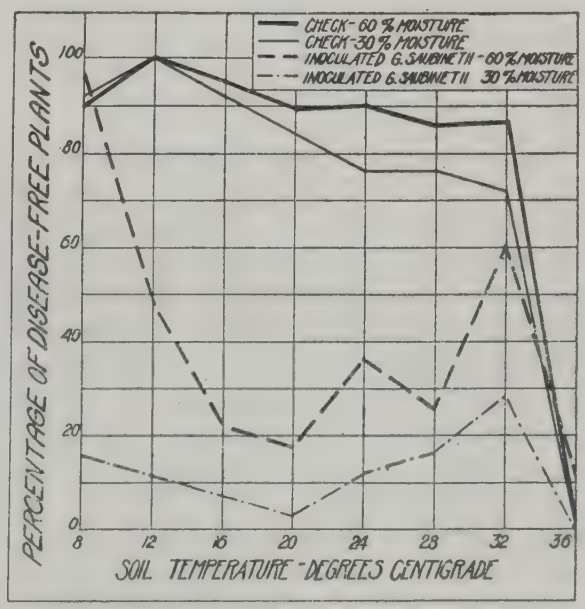


FIG. 9.—Graph showing relation of soil temperature and moisture to the development of seedling-blight of wheat. Compare the solid lines with the broken lines. Note the abundant blighting at 8° C. in the soil with a moisture content of 30 per cent of the moisture-holding capacity.

In order to study further the influence of moisture on the host and parasite two different temperature series of plants were grown at three different soil-moisture contents, 30, 45, and 60 per cent of moisture-holding capacity. The cans of soil at different moisture contents were prepared, inoculated, and sown as described in the previous experiments. The seed was inoculated with the heavy conidial suspension, 325,000 conidia per cubic centimeter, in order to get the maximum temperature and moisture response. The average percentage results of the two series are given in Table 8 and figure 9.

[7] Credit is gladly given to Mr. B. Koehler for conducting the earlier studies on the relation of soil moisture to seedling-blight.

TABLE VIII.—*Influence of soil temperature with varying degrees of soil moisture on the development of seedling-blight of wheat*

[Average of two experiments]

Average soil temperature.	30 per cent moisture-holding capacity.					45 per cent moisture-holding capacity.			60 per cent moisture-holding capacity.				
	Control.		Inoculated.			Inoculated.			Control.		Inoculated.		
	Percentage germinated.	Percentage disease-free.	Percentage germinated.	Percentage disease-free.	Percentage diseased.	Percentage germinated.	Percentage disease-free.	Percentage diseased.	Percentage germinated.	Percentage disease-free.	Percentage germinated.	Percentage disease-free.	Percentage diseased.
°C.													
8	96	96	88	16	72	100	56	44	90	90	96	96	0
12	100	100	96	12	84	80	24	56	100	100	96	48	48
16	100	96	92	8	84	72	20	52	95	95	86	22	64
19	88	84	80	4	76	52	20	32	95	89	88	18	70
23	76	76	72	12	60	96	8	84	95	90	84	36	48
27	92	76	60	16	44	88	24	64	92	86	84	26	58
31	92	72	64	28	36	100	32	68	87	87	96	60	36
35	36	0	64	0	[a] 56	48	12	[a] 12	0	0	44	12	32

[a] Balance killed by high soil temperatures.

In the control series the germination and number of disease-free seedlings at the two different soil-moisture contents, 30 and 60 per cent of the moisture-holding capacity, were the same at the lower temperatures. At the higher temperatures, however, the low soil moisture slightly inhibited germination and plant development.

In the inoculated series, on the other hand, the germination percentage of seedlings emerging and the percentage of blighted seedlings were greatly increased in the low soil moistures and even at the low soil temperatures. Soil moisture, then, became the limiting environmental factor to such an extent that even the resistance in the seedlings at the low temperatures was broken down. At the 8° C. soil temperature, for instance, 72 per cent of the seedlings grown at 30 per cent moisture were blighted, 48 per cent of them before emerging, while 44 per cent of the seedlings grown at 45 per cent moisture were blighted, and 24 per cent of these were blighted before emerging. This was in marked contrast to the results at 60 per cent moisture, where, as in the previous experiments, no blight occurred. The percentage of blighted seedlings was correspondingly increased by low soil moisture at all temperatures (Pl. 5).

Soil moisture, then, was another important factor in predisposing the seedlings to attack by *Gibberella saubinetii*. And when moisture was the limiting factor, it was impossible to obtain the true temperature influences.

TEMPERATURE AND MOISTURE EXPERIMENTS IN THE FIELD

The greenhouse experiments have demonstrated that wheat and corn seedlings become susceptible to the wheat-scab parasite only when under unfavorable environmental conditions, which may be a single factor, such as temperature, moisture, soil reaction, and others, or a combination of several of these factors. These experiments have shown, further, that the two hosts respond conversely to at least one factor,

namely, temperature. That is, wheat blights in a comparatively warm soil above 12° C., whereas, on the other hand, corn blights in a cool soil below 20° to 24°. Inasmuch as certain of these factors, especially temperature and moisture, play an important rôle in forming the field environment for both wheat and corn seedlings, a series of periodic field sowings were made at Madison, Wis., during the spring and fall of 1920 and the spring of 1921. These should point toward possible remedial measures for this and similar seedling diseases of the cereal crops as well as check against the greenhouse results.

A uniform plot of land was prepared for the spring sowings in the previous fall and reworked at the earliest possible date in the spring. It has happened in the past two years that early in March the soil has warmed and dried sufficiently to permit the installation of the thermographs and electrical resistance thermometer bulbs to obtain soil-temperature readings and to sow the first wheat. Following this the temperatures have gone down again during the remainder of March and most of April. It has been possible in this way, by watching the daily soil temperature and moisture, to make seedings at different intervals, especially a few days after a rain when moisture was well within the limits of plant response (fig. 2), which would throw the germination period of the grain at soil temperatures below or above the critical temperatures for seedling-blight. By this method it has been possible to check the constant soil-temperature studies in the greenhouse with fluctuating field temperatures.

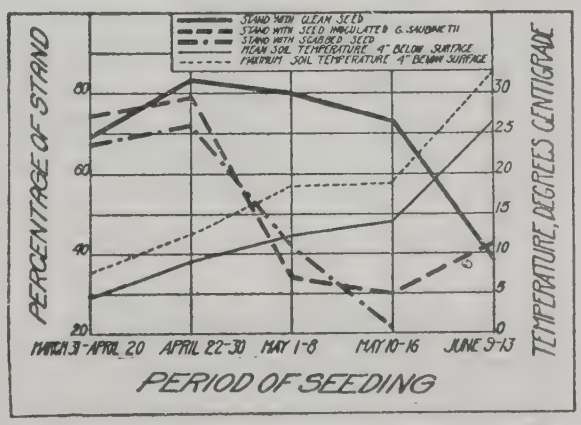
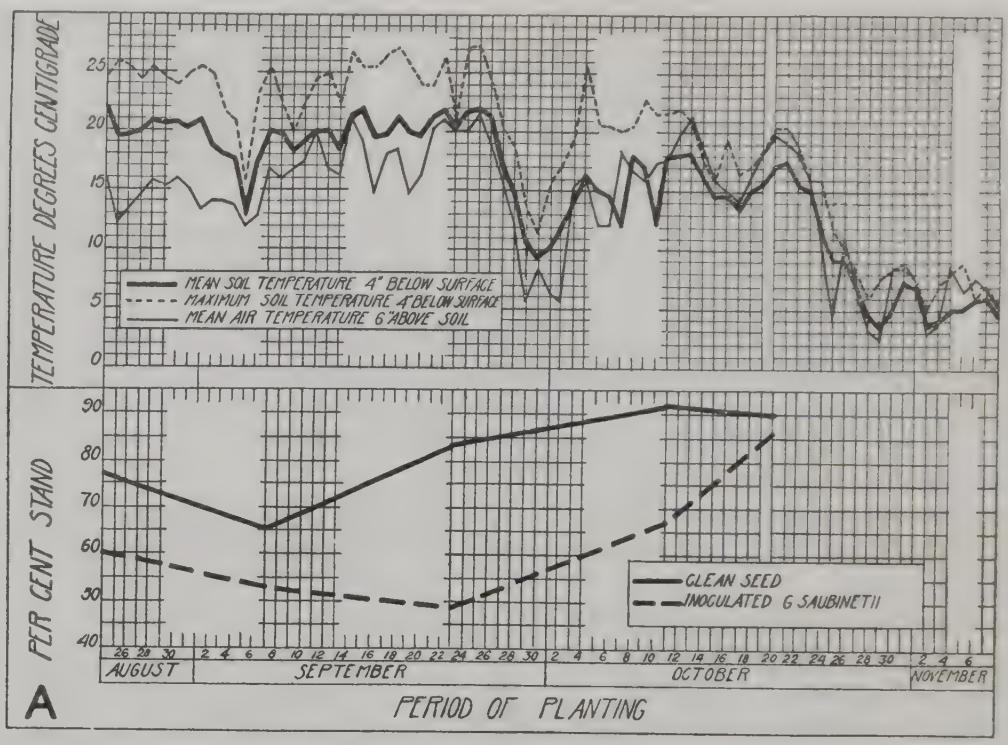


FIG. 10.—Graph showing relation of date of seeding to the development of seedling-blight of Marquis wheat in 1920. No blight occurred in the cool soil during March and April when the mean soil temperature was below 12° C. On the other hand, abundant blight developed in the later seedings, May 1 and 10, until the high soil temperatures of June inhibited the development of the parasite. (Summary of fig. 11.)

RELATION OF DATE OF SEEDING TO THE DEVELOPMENT OF SEEDLING-BLIGHT IN MARQUIS WHEAT

EXPERIMENT IN 1920

Two sowings were made on March 31 and April 22, which germinated before the average mean soil temperature was above 12° C. Following this, two sowings were made on May 1 and 10, which germinated with an average mean soil temperature between 12° and 15°. Finally, a sowing was made on June 9, during a period when the average mean soil temperature was about 26°, and the maximum running up to 36° for several hours during the middle of the day. Each sowing of these different periodic series consisted of 10 rod rows for control and inoculated seed and 5 rod rows for scabbed seed. The duration of the germination period, the percentage stand of disease-free plants, and the average mean temperatures are given in Tables IX and X and shown graphically in figure 10. The mean daily and daily maximum soil temperatures 4 inches below the surface and mean daily air temperatures 6 inches above the soil, together with the percentages of healthy plants in the different seedings, are given in figure 11.



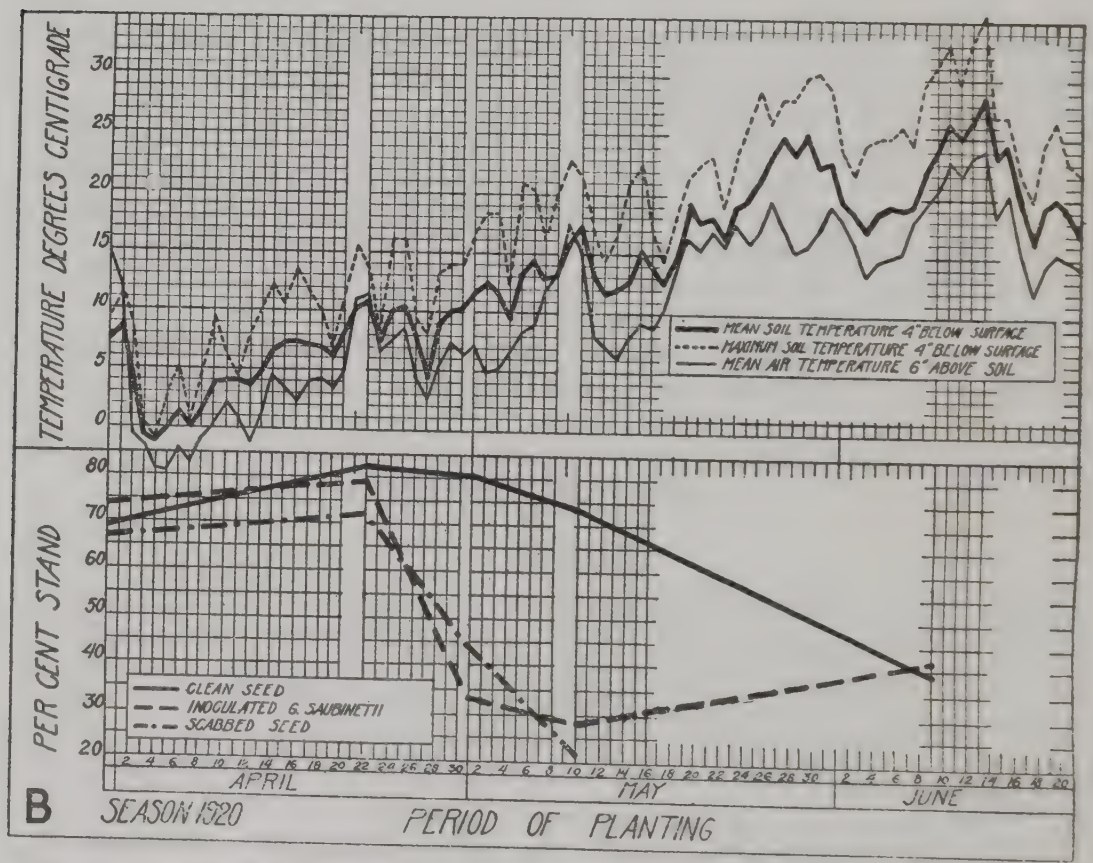


FIG. 11.—Graphs showing soil and air temperatures for the season of 1920 and percentage of stand of spring and winter wheat sown at different dates. A, Fall-sown Turkey wheat; B, spring-sown Marquis wheat. The squares of the graph paper are filled in for the seedling period of each sowing, as, for instance, August 24 to 29. This represents the period during which the enviromental factors influenced the development of seedling-blight. To avoid confusion the curves for percentage of stand of plants are plotted on the date sown. The distance between the solid lines and broken lines showing stand of control and inoculated plots represents the percentage of seeding-blight.

TABLE IX.—*Average soil and air temperatures during germination periods of Marquis wheat at Madison, Wis., in the spring of 1920*

Duration of germination period.		Average soil temperatures, 4 inches deep.			Average air temperatures, 6 inches above soil.		
Dates.	Number of days.	Mean.[a]	Min.	Max.	Mean.[a]	Min.	Max.
		°C.	°C.	°C.	°C.	°C.	°C.
Mar. 31 to Apr. 20	21	4.5	2.3	7.7	1.8	−1.9	6.3
Apr. 22 to 30	9	8.9	6.2	12.5	6.7	3.2	10.7
May 1 to 8	8	12.3	7.4	17.8	7.0	2.0	14.5
May 10 to 16	7	13.9	9.4	18.6	8.9	4.1	14.2
June 9 to 13	5	26.2	20.7	32.3	22.7	17.4	28.1

[a] Mean temperatures in this and succeeding tables were computed by adding together the temperatures recorded at hourly intervals during the germination period and dividing the sum by the number of hours in the period.

TABLE X.—*Relation of date of seeding to the development of seedling-blight of Marquis wheat at Madison, Wis., in the spring of 1920*

Date of seeding.	Average number of days for emergence.	Average percentage stand.		
		Clean seed not inoculated.	Clean seed inoculated with *Gibberella saubinetii*.	Naturally scabbed seed.
Mar. 31	22	69.0	74.2	66.5
Apr. 2	8	82.5	79.1	72.5
May 1	8	80.1	37.5	47.0
May 10	7	74.7	27.5	22.3
June 9	6	38.0	42.0	.....

The stands of healthy plants from the uninoculated, the inoculated, and the scabbed seed were practically the same for the first planting, which required 22 days to germinate—that is, the period from March 31 to April 20. The germination in all seed-lots was lowered by continued freezing and thawing during germination, but no blight appeared in any of the plots. The stand in the second series of seedings was greatly increased, due to more favorable weather during the germination period of 8 days. A limited number of blighted seedlings, however, appeared toward the last of the period in both the inoculated and scabbed series. The percentage of disease-free seedlings in the next two seedings was reduced 43 to 47 per cent, respectively, below the stand in the controls. The average mean temperature during the germination period and the daily temperature curves in figures 10 and 11 show a soil temperature below 12° C. for the first two seedings, and above 12° for the two May seedings. The critical soil temperature for seedling-blight in the field, therefore, corresponds very closely with that in the soil-temperature tanks.

The results from the last seeding, June 9, which was made during the extremely high temperatures, also agreed with the greenhouse results. That is, while germination was greatly reduced in both control and inoculated plots, no seedling-blight appeared, due probably to high temperatures inhibiting the development of the parasite.

The root systems of both the inoculated and control series of plants were excavated and studied at the maturity of the crop. Trenches were dug across the adjoining rows of the inoculated and control series in the March 31 and May 10 plantings, after which a gentle stream of water was used to wash the soil away from the roots. In this way a comparison was obtained, not only of the general root development but also of the root distribution. The development of lateral feeder root branches, the depth of root penetration, and the comparative root development in the two different seedings, as well as the influence of the fungus on reducing the root system, are shown in Plate 6, A, B, and C. The roots penetrated much deeper and developed more lateral branches in the early seedings

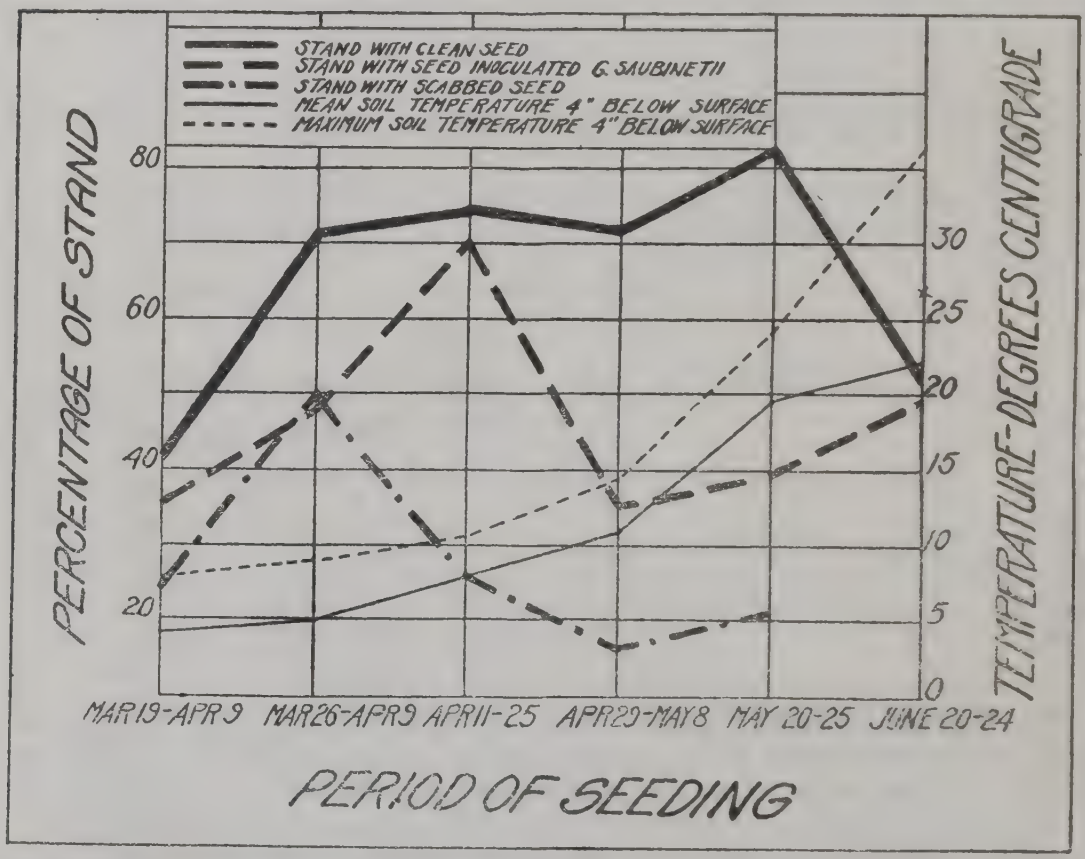


Fig. 12.—Graph showing relation of date of seeding to the development of seedling-blight of Marquis wheat in 1921. Results are essentially the same as in the previous season, shown in figure 10. (Summary of fig. 2.)

which were grown at a low soil temperature. Likewise, the parasite did not reduce the size of the root system at the low temperature, but the roots of the inoculated plants in the later seeding at high soil temperature were reduced nearly 50 per cent below those of the control plants.

EXPERIMENTS IN 1921

Three sowings made on March 19, March 26, and April 11, germinated before the average mean soil temperature for the germination period was above 12° C. The mean daily soil temperature for several days at a time during these three periods, however, was above 12°. The next two seedings, made on April 29 and May 20, germinated at an average mean soil temperature considerably above 12°, whereas the last seeding, on June 20, germinated during a fairly high mean soil temperature and fairly high maximum soil temperature.

The percentage of stand as well as the average mean soil temperature and mean daily soil temperature are given in Tables XI and XII and shown graphically in figures 2 and 12.

TABLE XI.—*Average soil and air temperatures during germination periods of Marquis wheat at Madison, Wis., in the spring of 1921*

Duration of germination period.		Average soil temperatures, 4 inches deep.			Average soil temperatures, 6 inches above soil.		
Dates.	Number of days.	Mean.	Min.	Max.	Mean.	Min.	Max.
		°C.	°C.	°C.	°C.	°C.	°C.
Mar. 19 to Apr. 9	21	4.6	2.8	7.8	7.3	2.4	12.4
Mar. 26 to Apr. 9	14	4.8	3.2	8.4	7.7	2.4	13.3
Apr. 11 to 25	14	7.7	5.4	10.7	8.9	4.8	12.9
Apr. 29 to May 8	9	10.5	6.5	14.7	8.5	3.5	13.5
May 20 to 25	5	19.6	15.4	23.9	20.8	15.4	26.4
June 20 to 24	4	22.5	19.6	26.7	21.0	17.2	27.7

TABLE XII.—*Relation of date of seeding to the development of seedling-blight of Marquis wheat at Madison, Wis., in 1921*

Date of seeding.	Average number of days for emergence.	Average percentage of stand.		
		Clean seed not inoculated.	Clean seed inoculated with *Gibberella saubinetii*.	Naturally scabbed seed.
Mar. 19	21	40.6	35.6	24.0
Mar. 26	14	71.6	47.6	49.6
Apr. 11	14	75.2	70.0	26.4
Apr. 29	9	71.7	35.8	17.8
May 20	5	83.0	39.6	21.2
June 20	4	52.0	49.2	.....

In general, the results in the second spring showed the same temperature responses as those of the previous year. The early seedings gave the highest stand of healthy plants, although some blight appeared in these plots, due probably to the fluctuating soil temperature and moisture. In all three germination periods of the first seedings the mean daily soil temperature was around or above 12° C. for several days at a time. Likewise, especially in the second germination period, the soil moisture was low enough to be an important factor in producing blight. The following two seedings both gave high percentages of blight, or, stating it reversely, a poor stand. The last seeding corresponded with the last seeding of the previous spring, which demonstrated that the attack of the parasite was probably checked by the high soil temperatures.

RELATION OF DATE OF SEEDING TO THE DEVELOPMENT OF SEEDLING-BLIGHT IN TURKEY WHEAT

The first sowings of winter wheat in 1920 were made in August when the soil temperature was fairly high. The fall was unusually warm, however, and the mean soil temperatures did not drop below 12° C. during September and early October, so that it was necessary to continue the seedings late into October to secure the low soil temperatures. All of the plots were sown when the temperature of the soil was above 12° C. except the last plot, sown on October 20. The percentage of stand and soil and air temperatures are given in Tables XIII and XIV and are shown graphically in figures 11 and 13.

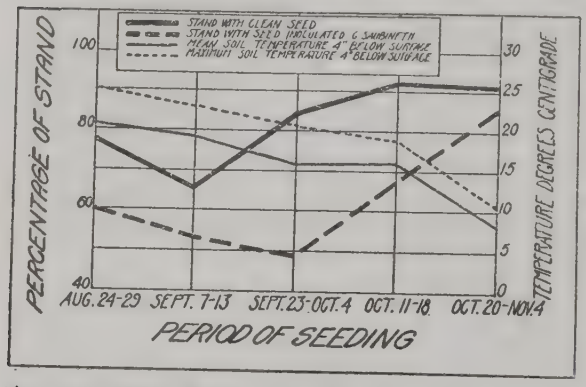


FIG. 13.—Graph showing relation o date of seeding to the development of seedling-blight of Turkey wheat in 1920. The cool soil temperatures of the late fall reversed the curves from those of figures 10 and 12. (Summary of figure 11.)

TABLE XIII.—*Average soil and air temperatures during germination periods of Turkey wheat at Madison, Wis., in the fall of 1920*

Duration of germination period.		Average soil temperatures, 4 inches deep.			Average air temperatures, 6 inches above soil.		
Dates.	Number of days.	Mean.	Min.	Max.	Mean.	Min.	Max.
		°C.	°C.	°C.	°C.	°C.	°C.
Aug. 24 to 29	6	20.4	16.3	25.0	14.4	10.0	21.5
Sept. 7 to 13	7	19.2	15.4	23.5	17.2	11.6	23.1
Sept. 23 to Oct. 4	12	15.8	12.3	20.2	13.9	8.4	20.3
Oct. 4 to 18	9	15.9	14.3	18.7	17.4	13.2	20.8
Oct. 20 to Nov. 4	16	8.2	6.8	11.3	9.5	6.0	13.7

TABLE XIV.—*Relation of date of seeding to the development of seeding-blight and winter-killing of Turkey wheat at Madison, Wis., in the fall of 1920*

Date of seeding.	Average number of days for emergence.	Average percentage of stand, fall, 1920.		Average percentage of stand, spring, 1921.		Average percentage of winter killing.	
		Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.
Aug. 24	6	77.0	60.0	68.0	48.8	9.0	11.2
Sept. 7	7	55.0	53.0	44.0	48.2	11.0	4.8
Sept. 23	12	83.5	49.5	56.0	31.6	27.5	17.9
Oct. 9	9	92.0	67.0	49.2	29.2	42.8	37.8
Oct. 20	16	90.0	86.0	71.7	57.8	18.3	28.2

The first two sowings showed the influence of high soil temperature upon both host and parasite. The germination and stand of the controls was greatly reduced, yet the reduction in germination and stand from blight was not as great as in the following two sowings where the mean soil temperature was 16° C. The last seeding, however, in which the seed germinated in a cool soil, gave little blighting, and, further, the stand and general vigor of the plants were comparable to the condition of the controls.

These soil-temperature experiments in the field have shown, then, that wheat germinated in a cool soil resists the blight but when germinated in a warm soil succumbs to the attack. These results also have shown that the critical soil temperature for the production of the seedling-blight of

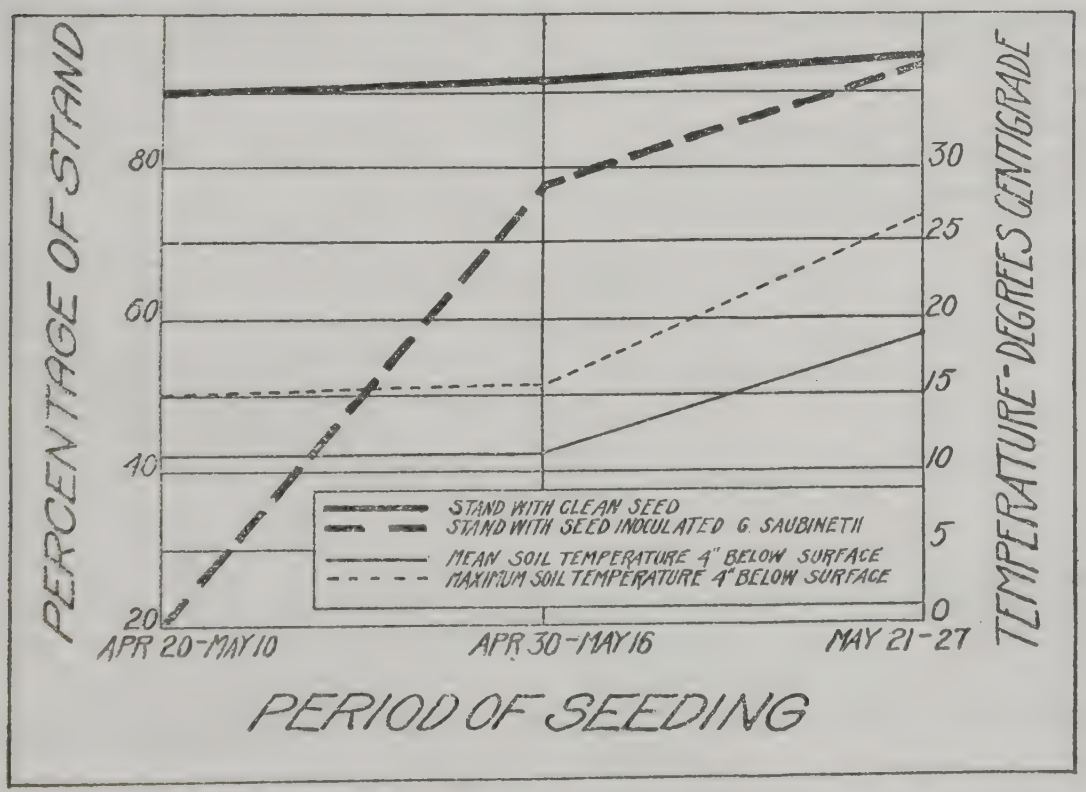


FIG. 14.—Graph showing relation of date of planting to the development of seedling-blight of corn in 1921. The seedling-blight occurred during the April plantings when the mean soil temperature was below 20° C. No blight developed when the mean soil temperature reached 20° C. (Summary of fig. 2.)

wheat is the same under field conditions as in the soil-temperature tanks in the greenhouse, namely, about 12° C.

These results already have been applied as control measures by agronomists and other wheat growers, yet further emphasis is necessary from the phytopathological viewpoint. Seeding at the earliest safe date in the spring for spring wheat and the latest safe date in the fall for winter wheat is the best method of reducing the seedling-blight. Agronomists have shown that the date of planting has a marked relation to lodging and to yield and quality of grain. The marked reaction between the date of seeding and the root development may partly explain those relations.

RELATION OF DATE OF PLANTING TO THE DEVELOPMENT OF SEEDLING-BLIGHT IN CORN

The general field observations during the past two seasons have demonstrated beyond question the influence of soil temperature upon the seedling-blight of corn and, in themselves, have corroborated the soil-temperature studies in tanks in the greenhouse. A preliminary series of periodic planting experiments with corn was started in the spring of 1921 to check more exactly the soil-temperature tank results. The first planting, April 20, germinated when the mean soil temperature was about 12° C.; the second planting, April 30, germinated a few days before most of the corn was planted in the vicinity of Madison. While the average mean soil temperature in this period was about the same as during the previous period, the daily mean temperature, as shown graphically in figures 2 and 14, did not run as low at any single period as in the previous germination period. The last planting, May 31, germinated in a soil at about 20°. The percentage stand of plants for the three periods and temperatures are given in Tables XV and XVI and shown graphically in figures 2 and 14.

TABLE XV.—*Average soil and air temperatures during the germination periods for corn at Madison, Wis., 1921*

Duration of germination period.		Average soil temperatures, 4 inches deep.			Average air temperatures, 6 inches above soil.		
Dates.	Number of days.	Mean.	Min.	Max.	Mean.	Min.	Max.
		°C.	°C.	°C.	°C.	°C.	°C.
Apr. 20 to May 10	20	11.7	8.1	15.2	10.8	6.3	15.5
Apr. 30 to May 16	16	11.7	8.0	15.5	9.7	4.8	14.4
May 21 to 27	6	18.8	15.5	26.8	20.1	15.7	25.2

TABLE XVI.—*Relation of date of planting to development of seedling-blight of corn at Madison, Wis., in 1921*

Date of planting.	Average number of days for emergence.	Average percentage of stand.	
		Disease-free seed.	Disease-free seed inoculated with *Gibberella saubinetii*.
Apr. 20	20	90	20
Apr. 30	16	91	78
May 21	6	94	93

The first planting was most severely affected by blight, 70 per cent of the plants being killed during the seedling stage. Most of these were blighted during germination or just before emergence. The mean daily soil temperature, as shown graphically in figure 2, contributed directly to this blighting. The soil temperature following sowing was

high enough to start immediate germination. This was followed in 8 days by a low temperature period of about 10 days' duration in which the temperature went considerably below the critical temperature for blighting.

The second planting was made during the low-temperature period, but did not begin germinating until the soil temperature had risen to about 16° C. While this planting was subjected to low soil temperatures later in the germination period, the soil temperatures did not go as low as those following the previous planting, and the depression occurred later in the period of seedling development. As a result, the stand was reduced only 13 per cent. The last planting showed only a small degree of blighting in two or three of the most susceptible strains.

The field temperature experiments with corn in general showed results similar to those obtained in the greenhouse. The field plantings, however, indicated a slightly lower critical temperature for blighting than that shown in the soil temperature tanks in the greenhouse.

DISCUSSION OF RESULTS

While the influence of soil temperature in the development of seedling-blight was operative over a wide range of temperatures, there was, however, in both wheat and corn a very clearly defined critical temperature for blighting. Soil temperatures below 12° C., for instance, prevented blighting of the wheat seedlings, whereas soil temperatures at 12° or above produced a large amount of blight. The critical soil temperature, therefore, for the seedling-blight of wheat was always at 12° C., whether a constant soil temperature or the mean of a fluctuating field soil temperature. The critical soil temperature is represented graphically in Figures 5 and 6 for the constant soil temperature series and in Figure 15 for one instance in the field series. Other cases equally striking could be chosen from periodic seedings of either the spring or the winter wheat.

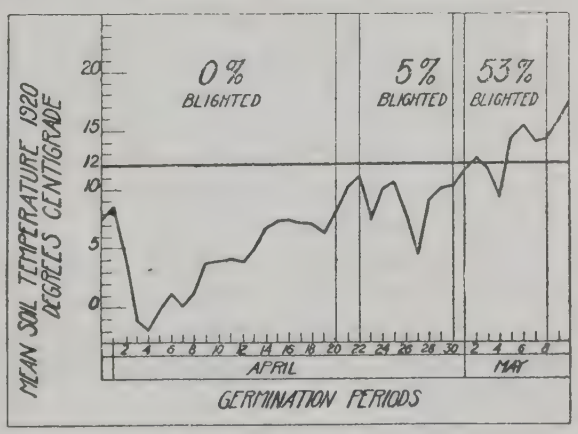


FIG. 15.—Graph showing critical soil temperature for the development of seedling-blight of wheat. The heavy horizontal line at 12° C. represents the critical soil temperature for the development of blight in the soil-temperature tanks in the greenhouse. The rise of the daily mean soil temperature to and above the 12° C. line resulted in the development of seedling-blight.

The straight line at 12° C. in figure 15 represents the approximate critical soil temperature for seedling-blight of wheat. The first seeding germinated and emerged when the soil temperature curve was well below this line, with no blight as a result. The second sowing developed in a warmer soil with a mean temperature still below the 12° line, yet approaching it. In many cases the temperature during a portion of the day was above this line. The blight appeared on just a few of the seedlings. The last seedings shown developed in a soil having a mean temperature of 12° or above, and as a result 53 per cent of the plants were blighted. The mean soil temperature in fluctuating field temperatures corresponded very closely with the constant soil temperatures in the greenhouse experiments.

The seedling-blight of corn, both in greenhouse tank series and in field series, developed at 20° to 24° C. and below, while above 24° no

blight occurred. The critical soil temperature for the development of of seedling-blight of corn, therefore, is about 20° (fig. 7). Here, again, the mean daily soil temperature in the field corresponded very closely with the constant soil temperature in the tanks in the greenhouse. These facts seem rather significant to the understanding of the relation of environment to the susceptibility of the hosts to this disease. They indicate, at least, that in this type of disease soil temperature is important as an environmental factor, and, further, that mean soil temperatures over periods of considerable duration are more influential as factors than brief extremes in soil temperature.

SUMMARY

(1) The seedling-blight of wheat caused by *Gibberella saubinetii* is manifest first by blighting before emergence, which results in poor germination, second by blighting after emergence, which is evident by the yellowed and wilted seedlings, and third by stunting the seedling which is due to the weakened root system.

(2) The symptoms of the seedling-blight of corn, caused by this same organism, are, in general, similar to those of wheat.

(3) The invaded tissues in both wheat and corn become reddish brown to carmine red, depending upon environmental factors.

(4) The disease develops from infected seed, scabbed seed in the case of wheat, and infested soils.

(5) The period of seedling infection in both wheat and corn is usually limited to the early seedling stage.

(6) With environmental factors favorable, the extent of blighting and injury from seedling-blight is directly proportional to the amount of infestation.

(7) The parasite in pure culture functions normally over a comparatively wide range of temperature, namely, from 3° to 32° C. The optimum temperature for spore germination, vegetative development, and sporulation is about 24° on unacidified and 28° on acidified media.

(8) Wheat is a low-temperature plant and functions best at a low soil temperature in all stages of its development. On the other hand, corn is a comparatively high-temperature plant and develops best at high soil temperatures.

(9) The temperature of the soil is undoubtedly the most important single factor determining the extent of seedling-blight. The most favorable soil temperature for the blighting of wheat ranges from 12° to 28° C. Below 12° no blight occurred if other factors were favorable. In contrast, the most favorable soil temperature for the blighting of corn ranges from 8° to 20°. Above 24° no blight occurred.

(10) Low soil moistures favor blighting of wheat even at low soil temperatures.

(11) Results from sowings of wheat and corn made at different dates under field conditions agree with those from the greenhouse experiments. Seeding when the soil is cool, that is, spring wheat at the earliest safe date in the spring and winter wheat at the latest safe date in the fall, reduces seedling blight. On the contrary, seeding corn when the soil is warm, that is, at the latest safe date in the spring, reduces seedling-blight.

(12) There are sharp critical temperatures for the production of the blight of both wheat and corn. The mean daily soil temperature in the field corresponds to the constant soil temperature in the soil-temperature tanks. The critical soil temperature for the seedling-blight of wheat is

about 12° C., in both constant soil temperature and mean daily field soil temperature. The critical soil temperature for the seedling-blight of corn is between 20° and 24° under both greenhouse and field conditions.

(13) Infection takes place and the blight progresses, then, at entirely different temperatures with the two different hosts, wheat and corn, inoculated with the same parasite. Hence, the influence of environmental factors on the hosts seems to be the fundamental cause of susceptibility to the disease. The seedlings become susceptible when they are unable to respond favorably to the environment.

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PLATE 1

Seedlings and kernels of Marquis wheat attacked by the wheatscab fungus, *Gibberella saubinetii*.

Seedlings blighted after inoculation in the field with conidia of the parasite grown in pure culture. *a*, Killed before emerging; *b*, killed just after emerging; *c*, killed in first-leaf stage; *d*, badly scabbed kernels, shrivelled and dead; *e*, slightly scabbed, discolored kernels only slightly, if at all, shrunken, but which produce infected seedlings when they germinate; *f*, sound, plump kernels for comparison. ×2.

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PLATE 2

Seedlings of Marquis wheat, (*a*) infected by *Gibberella saubinetii* and (*b*) uninfected, both of the same age.

The infected seedling was from the same inoculation series as those shown in Plate 1, *a*, *b*, and *c*. Note marked reduction in vigor, browning of basal portion, and reduction of root system as well as yellowing of leaves and absence of tillers. The uninfected seedling was from a corresponding uninoculated series. Note general vigor, clean basal portion, abundant vigorous roots, as well as vigorous, healthy leaves and tillers. Natural size.

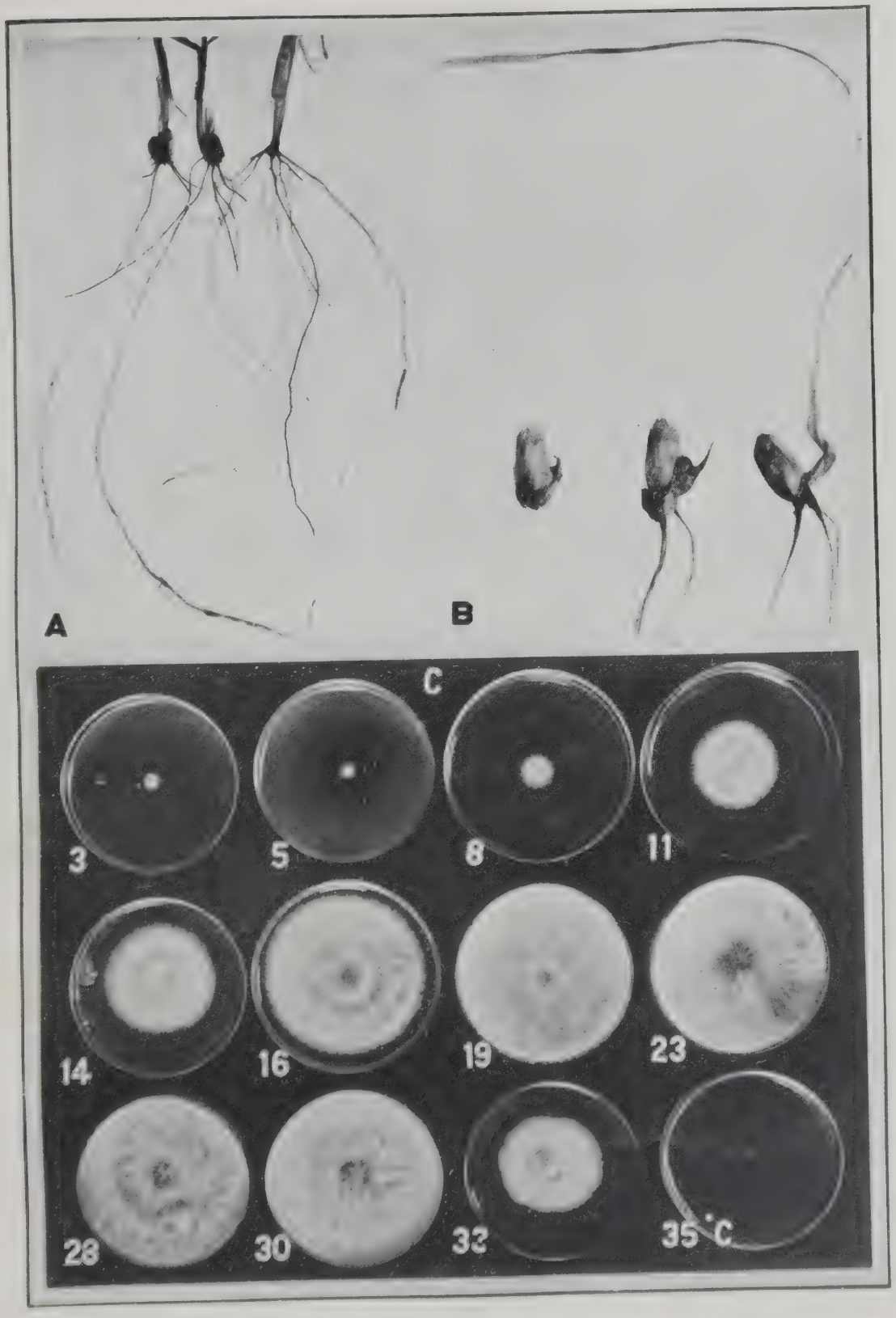
PLATE 3

Wheat seedlings attacked by wheatscab fungus, *Gibberella saubinetii*, and Petri-dish cultures of the same fungus incubated six days at different temperatures.

A. Winter wheat (Turkey) seedlings from artificially inoculated seed, taken from the field late in the fall. The blackened, rotted roots resulted from the progress of the disease early in the season while the soil was warm. The new, white, healthy roots developed later in the season while the soil was cool.

B.—Blighted seedlings of Marquis wheat from artificial inoculation, taken from the field in the spring. The first two seedlings (left) represent typical blighting before emergence. The seedling on the right was blighted in the first-leaf stage.

C.—Relation of temperature to the vegetative development of *G. saubinetii*. Petri-dish cultures of the fungus incubated six days on unacidified, potato-dextrose agar at temperatures from 4° to 35° C., as indicated by figure below each culture.


A
B
C
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5
8
11
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16
19
23
28
30
32
35°C

1
2
A
1
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B
1
2
C
1
2
D

PLATE 4

Representative corn seedlings, from inoculated and uninoculated seed, grown at different soil temperatures.[1]

A.—Plants grown at a soil temperature of 12° C. Plants from the inoculated seed were all killed before emerging.

B.—Plants grown at a soil temperature of 16° C. Plants from the inoculated seed were badly diseased. About half of them blighted soon after emerging.

C.—Plants grown at a soil temperature of 20° C. Plants from the inoculated seed almost as badly diseased as those grown at 16° C., represented in B.

D.—Plants grown at a soil temperature of 24° C. Plants from the inoculated seed were healthy except for a few minor root lesions.

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[1] In each set of plants from A to D, (1) represents typical plants from seed inoculated with conidia of *Gibberella saubinetii* and (2) represents seedlings of the same age and from the same seed uninoculated.

PLATE 5

Influence of soil temperature and moisture on the development of seedling-blight of Marquis wheat.[1]

A.—Seed sown in soil wet to 30 per cent of its moisture-holding capacity. Note percentage of blight which developed at 8° C.

B.—Seed sown in soil wet to 45 per cent of its moisture-holding capacity. Note decrease of blight which developed at 8° C.

C.—Seed sown in soil wet to 60 per cent of its moisture-holding capacity. No blight developed at 8° C., and less at all temperatures than in the drier soils.

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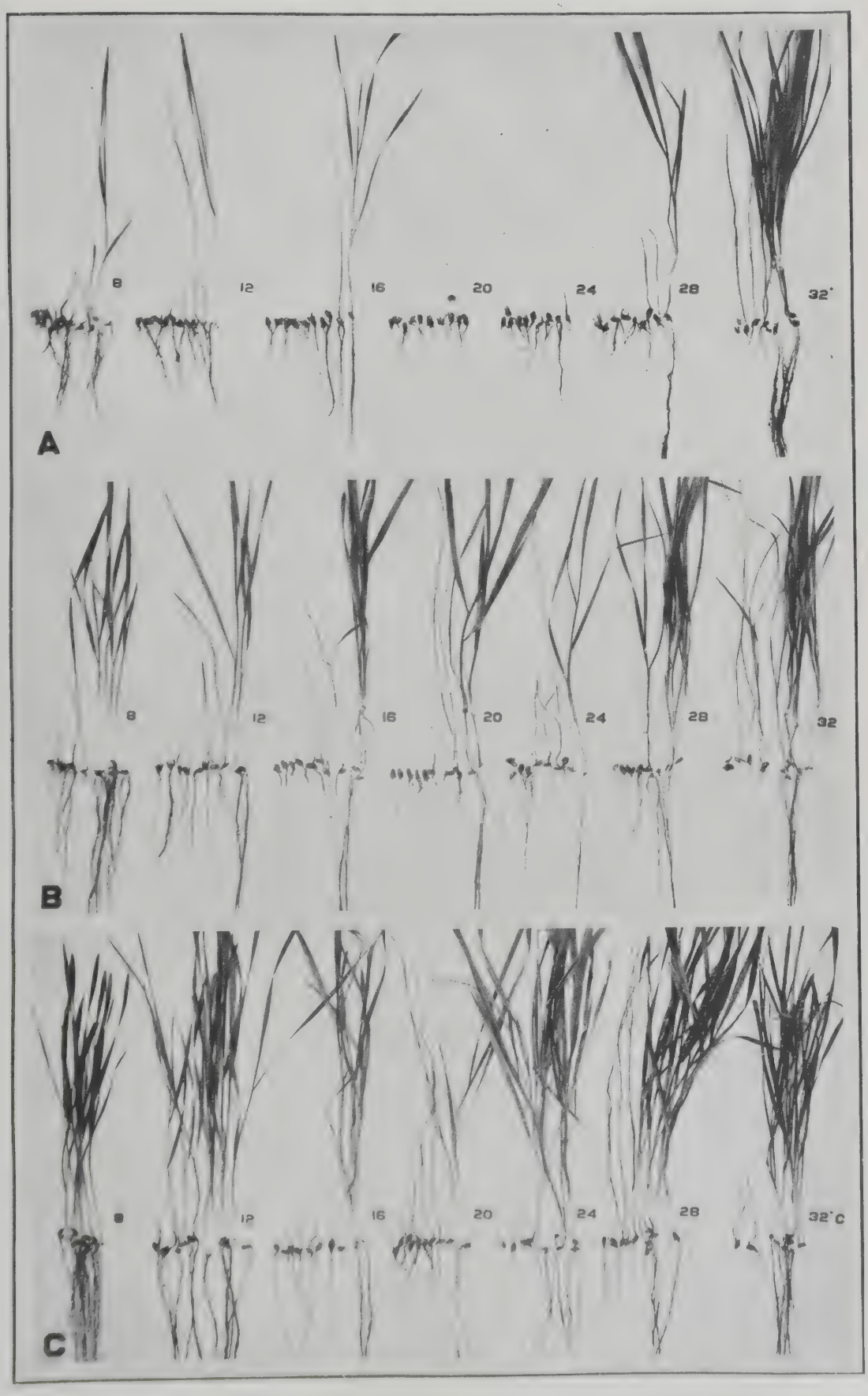
[1] The 10 seedlings in each group represent the proportion of healthy and blighted seedlings from a series of 200 plants grown at each soil temperature from seed inoculated with *Gibberella saubinetii*.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APR 22
A
MAY 11
B
I
II
E
C
D

PLATE 6

Relation of date of seeding to the development of seedling-blight of Marquis wheat.

A.—Wheat plants from inoculated, scabbed, and clean seed sown April 22, when the soil temperature was below 12° C. Note the uniformly good stand from all three lots of seed.

B.—Wheat plants from inoculated, scabbed, and clean seed sown May 10, when the soil temperature was above 12° C. Note the greatly reduced stand from the inoculated seed and especially from the scabbed seed.

C.—Root development of wheat sown April 22. Left, uninoculated; right, inoculated with *Gibberella saubinetii*. Note the large root systems in each.

D.—Root development of wheat sown May 10. Left, uninoculated; right, inoculated with *G. saubinetii*. Note that the root development is much less extensive than on the plants from the April 22 seeding shown in C. See E for the detailed comparison of the root systems.

E.—Root systems of the four lots of spring-wheat plants shown in position in C and D. Each bundle contains the roots of five plants. (1) Sown April 22; left, uninoculated; right, inoculated. (2) Sown May 10; left, uninoculated; right, inoculated. Note the reduction in root systems in (2) right, inoculated seed sown May 10, when the mean soil temperature was above 12° C. In contrast, note the lack of reduction in (1) right, where the seed was inoculated and sown April 22, when the soil temperature was below 12° C.

FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT [1]

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INTRODUCTION

In an earlier paper (*7*)[2] considerable data were presented tending to show that the length of day exercises a remarkable regulatory action in initiating or inhibiting sexual reproduction in plants. In a number of species studied it was found that ordinarily the plant can attain the flowering and fruiting stages only when the length of day falls within certain limits, so that in such cases flowering and fruiting occur only at certain seasons of the year. In this respect some species and varieties respond to relatively long days while others respond to short days. Moreover, some plants are much more sensitive to change in length of day than are others. In the absence of the particular day length favorable to sexual reproduction vegetative development may continue for a more or less indefinite period, thus frequently leading to the phenomenon of gigantism. It was discovered, also, that exposure to a daily light period intermediate between that favorable only to vegetative development, on the one hand, and that favoring only flowering and fruiting on the other hand, tends to cause both forms of activity to progress simultaneously. This combined form of activity constitutes what is commonly known as the "everflowering" or "everbearing" behavior. It was suggested that probably the seasonal range in length of day is an important factor in the natural distribution of plants and that in agricultural practise the correct time for planting many crop plants may be largely conditioned by the prevailing length of day. To designate the response of the plant to length of day the term "photoperiodism" was suggested.[3] The data on which these conclusions are based were obtained chiefly by use of dark chambers into which the test plants could be placed for a portion of the day, thus reducing the number of hours of illumination received during the relatively long days of summer. Brief mention also, was made of greenhouse experiments conducted during the winter months, in which electric light was used to prolong the daily period of illumination. The results of these latter tests confirmed those previously obtained by use of the dark chambers. Oakley and Westover (*18*) have recently reported an interesting application of these principles as a means for the quick identification of seed of different alfalfas.

[1] Accepted for publication May 10, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 919–920.

[3] The authors are indebted to Mr. O. F. Cook, of the Bureau of Plant Industry, for suggesting the term photoperiodism, which seems to meet satisfactorily all requirements as to both aptness and simplicity.

In connection with the discussion of the literature bearing upon the subject which was presented, attention is here called to an article by Klebs (*10*) on flower formation in Sempervivum, published in 1918, which had not become accessible to the writers at the time the previous paper was written. Following methods somewhat similar to those previously employed by Bonnier, Bailey, Rane, and Corbett, as briefly outlined in our former paper, Klebs showed that Sempervivum, which normally flowers in June, could be forced into flowering in winter, either by means of continuous electric illumination for a few days or by increasing the daily illumination period to more than 12 hours. The Osram lamp was used both for continuous illumination and for daily illumination periods of different duration. This result with Sempervivum is essentially the same as that obtained by Bailey and by Rane with spinach (*Spinacea oleracea* L.), but Klebs, perhaps, made a more complete analysis of the response and his data are more nearly quantitative in character. He also studied the effects of change in intensity and quality of the light on flowering in Sempervivum. Light seems to be essential for the formation of flower primordia. The response of Sempervivum to differences in light exposure is interpreted by Klebs as supporting the theory previously advanced that flowering is the result of increased concentration of the cell sap, particularly with respect to carbohydrates. In summarizing, it is stated that in flowering the quantity of the light energy is of decisive importance.

It is especially desirable, also, to call attention here to the remarkable life histories of two small marine worms, *Convoluta roscoffensis* and *C. paradoxa*, as worked out by Keeble (*9, p. 31*) and others. A curious form of symbiosis with a green and a brown alga, respectively, is involved, so that these creatures have received the designation "plant animals." The movements of these worms, which live on the seashore, correspond with the incoming and outgoing tides. Thus, *C. roscoffensis* regularly sinks below the surface of the sand with the incoming tide and emerges as the tide recedes. This synchronism with tidal movement has been shown to be due to the alternate exposure to light and darkness as the tide recedes and again advances. What is of special interest here, however, is the remarkable fact that with this organism reproductive activities occur chiefly at 2-week intervals, beginning with the onset of spring tides. This is due to the fact that at this season of the year low water occurs only once during the day, the other period coming at night, with the result that the worms receive daily only about six hours of sunlight. Egg-laying reaches its maximum when the animals are subjected daily to one short spell of six hours' light-exposure followed by a long spell of eighteen hours' dark exposure. But—and the fact is remarkable—these conditions of light and darkness are precisely those to which *C. roscoffensis* is exposed during the spring tidal periods at which its eggs are laid habitually.

The observations and conclusions recorded in the former paper concerning the regulatory action of the relative length of day and night on flowering and fruiting as against the purely vegetative form of activity have been confirmed and considerably extended by further study of the subject, as will be outlined in this paper. In view of the marked influence of day length on sexual reproduction it seemed likely that plants would be found to respond to this influence in other ways, and, accordingly, a series of experiments was made to test this possibility. It was found, in fact, that the plant is capable of responding in many ways to change in the duration of the daylight period. Thus, most if not all outstanding

phenomena of periodicity seem to be more or less influenced by the seasonal change in length of day. Moreover, some features of plant development not so definitely periodic in character appear to be related to the duration of the daylight period. Because of the wide field covered a great deal of experimental data must be accumulated before a full discussion of these various responses can be undertaken, and it is not practicable at this stage to attempt to correlate the extensive literature on the different features of plant development with the preliminary observations which have been made. In the present paper a somewhat more detailed discussion of the formation of tubers, bulbs, and thickened roots in relation to sexual reproduction will be undertaken. Only briefer mention of other features of the photoperiodic response will be made, reserving fuller consideration for the future. Perhaps the principal value of the limited number of observations which have been made on various forms of response, viewed as a whole, is that they may be made the basis of a working hypothesis as to the nature of some of the internal processes involved in photoperiodism, thus suggesting more systematic biological and biochemical studies along definite lines. The wide extent and great variety in form of the photoperiodic response verifies in most striking manner the soundness of the modern view, the most consistent supporter of which perhaps has been Klebs, to the effect that environment through its action on internal conditions governs the form of expression in the plant, subject only to the specific limitations imposed by heredity.

FURTHER OBSERVATIONS ON FLOWERING AND FRUITING

Woody perennials, particularly the larger-growing forms, do not lend themselves so readily to the methods of study which have been followed, so that the data thus far accumulated have reference mostly to annuals and herbaceous perennials, though a number of shrubs and dwarf trees are now under observation. In the following experiments the methods followed were essentially the same as those described in the former paper; namely, the use of light-proof houses into which the plants could be placed for a portion of the day during the open growing season and the use of electric light in the greenhouse for a portion of the night during the winter months. The average intensity of the electric illumination maintained in the vicinity of the plants in the greenhouse was approximately 3 to 5 foot-candles and the lights (40-watt mazda bulbs) were on from sunset till midnight each day. In reducing the daylight period the plants in most experiments received sunlight only from 10 a. m. to 3 p. m. for the 5-hour exposures; from 5.30 a. m. to 3.30 p. m. for the 10-hour exposures; from 5.30 a. m. to 5.30 p. m. for the 12-hour exposures; from 5.30 a. m. to 6.30 p. m. for the 13-hour exposures.

Seed of *Amaranthus hybridus* L. which was sown in metal buckets July 8 had germinated by July 11. Under daily light exposures of 10, 12 and 13 hours the first blossoms opened August 6 while the average heights of the plants, 20 to 30 in number, were 13 to 14 inches, 16 to 17 inches, and 15 to 16 inches, respectively. With 5 hours of light daily, first blossoms were open August 14 and the average height was 8 to 9 inches. The controls exposed to the full natural day length first showed blossoms August 23 and the average height was 26 to 27 inches.

Kulthi bean (*Dolichos biflorus* L.), planted May 29, showed open blossoms July 21 under a 13-hour daylight exposure. Flowering was sparse and temporary, followed by rapid growth. On September 1 flower buds were again evident. The controls first showed open blossoms August 2 and also were again showing flower buds on September 1. It appears that a day length somewhat less than 13 hours is required for quick and profuse flowering in this species.

Peruvian flour corn (*Zea mays* L.), supplied by the Office of Cereal Investigations, Bureau of Plant Industry, was planted May 29 in boxes 10 inches square and 3 feet long. Under a daylight period of 13 hours the plants were shedding pollen July 26 and under natural daylight conditions on August 24. The respective heights of the plants were 71 and 92 inches.

A very late-maturing variety of corn from Mexico, known as Tuxpan, planted May 22 in boxes as above, was shedding pollen August 6 as a result of receiving a 10-hour light exposure and the average height was 50 inches. Under a 13-hour light exposure pollen was falling August 14 and the average height was 53 to 54 inches. The controls were shedding pollen September 13 and the height was 86 inches. It is worthy of note that at the middle of September the 10-hour day plants, as well as the controls, were decidedly greener in color than those exposed to a 13-hour day.

Tests were made with several sorghums (*Holcus halapensis* L.) from tropical Africa and India, seed of which was furnished by the Office of Forage Crop Investigations, of the Bureau of Plant Industry. Seed was sown June 17. The pots containing the plants were transferred to the greenhouse October 11 to prevent injury from cold weather. The results of the tests are summarized in Table I.

In the former paper (7) the behavior of buckwheat (*Fagopyrum vulgare* Hill) in the greenhouse in winter was briefly described. Under the natural day length the plants completed the life cycle within a few weeks, attaining a relatively uniform height of only 24 inches. Increase in the duration of the daily light period to about 18 hours with electric light gave marked irregularity in height attained and degree of flowering. Some individuals attained a height of 9 feet or more, and all acted as typical ever-bloomers, with indeterminate growth characteristics. Repetition of the experiment in summer (seed planted May 18) gave similar results except that the height attained by the plants under the longer natural day length was increased to between 4 and 4½ feet. Outdoor tests also were made in the summer under regulated day lengths of 5, 10, and 13 hours, with controls, all plantings being made June 8. Flowering began in the controls on July 6; in all other cases on June 30. Under a daylight period of 5 hours the plants were very small and soon died, after having produced a few seeds. With 10 hours of light daily the plants lived longer and attained a height of 24 inches. Under the 13-hour exposure the plants were slightly taller and more seeds were produced. The controls averaged 36 inches in height and fruited freely. Thus, it is easily seen why buckwheat makes best growth in high latitudes.

TABLE I.—*Effect of length of day on growth of sorghums from tropical Africa and British India*

S. P. I. varietal number.[1]	Point of origin.	Number of plants grown.			Date of shedding first pollen.			Average height August 17 (inches).			Stooling or branching, August 17.		
		10-hour day.	13-hour day.	Control.	10-hour day.	13-hour day.	Control.	10-hour day.	13-hour day.	Control.	10-hour day.	13-hour day.	Control.
37114	Kano, North Nigeria, lat. 12° N.....	5	3	4	Aug. 2	Nov. 29	Dec. 14	52	30	27	Marked.	Slight.	None
39449	Calulo, Angola, lat. 15° S.....	12	6	5	3	.....	14	43	33	23	None...	None..	Do.
39451	Calulo, Angola.....	5	5	5	4	Dec. 17	17	52	33	26	...do....	...do...	Do.
43626	Caunpore, India, lat. 20° N.....	5	3	3	9	.....	Sept. 25	65	66	40	...do....	...do...	Do.
30450	Togo, German West Africa, lat. N. 8°..	5	9	7	2	Oct. 23	Nov. 15	70	42	34	Marked.	...do...	Do.
39450	Calulo, Angola.....	4	5	5	3	Dec. 15	Dec. 17	54	32	27	None ..	...do...	Do.

[1] The varietal numbers are those of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry.

Roots of perennial wild sunflower (*Helianthus giganteus* L.) transferred to the greenhouse in October soon developed vigorous shoots. Under natural daylight conditions flower buds appeared early in April and open blossoms in May. With the natural daylight period lengthened by use of the electric light till May 26, flowering was inhibited and there were no indications of blossoming as late as July 1.

Another wild sunflower, *Helianthus angustifolius* L., an eastern and southern species, was planted in the electrically lighted house January 27. Under the long daily light exposure the plants grew to a height of 6 to 7 feet without flowering at any time during the year. Specimens transferred to a daily light exposure of 10 hours, beginning September 2, began flowering October 4 (Pl. 2, A). Another lot of the plants exposed to the natural day length, beginning September 2, also flowered as a result of decreasing day length but much later than those under the 10-hour exposure. The plants remaining in the electrically illuminated house finally formed flower buds in November, but under the combined influence of the weak electric light at night and the natural light of the short winter days these buds were prevented from unfolding and remained in a practically dormant condition throughout the winter.

Cosmos bipinnata Cav., planted November 1, was in flower December 22 and attained a final height of 30 inches under the normal day length. Continuing the light period till midnight by means of the electric light until arrival of the long summer days inhibited flowering. Growth continued until early October, and the plants had attained a height of 15 feet when forced into flowering by the decreasing day length.

Klondike cosmos (*Cosmos sulphurea* L.) was planted April 22 and had germinated April 25. Under a 10-hour day beginning July 6 flower buds were showing July 19, and the first blossoms were open August 1. The control plants exposed to the natural length of day first showed flower buds October 1, and the first open blossoms appeared October 14.

Corms of blazing star (*Liatris graminifolia* Walt.) were transplanted to metal buckets March 27. Under a 10-hour day the first blossoms opened

June 21, the plants averaging 17 inches in height. Under a 13-hour day blossoming began July 22, and the average height was 24 inches. The controls began flowering August 26 and were slightly taller than the 13-hour day plants. In the following season a second lot of corms were transplanted from the field May 19 and exposed to different day lengths. Under a 10-hour day flower buds were showing June 6, and the first open blossoms appeared July 2. The average height of the plants was 12 inches. Under a 12-hour day flower buds could be seen June 6, and the first blossoms unfolded June 30, when the plants had attained a height of 12 inches. The controls were showing flower buds July 22 and first open blossoms September 12. The average height was 35 inches.

Tithonia rotundifolia (Mill) Blake, planted January 4, was in bloom March 14 under natural daylight, having attained a height of 48 inches. In the greenhouse electrically lighted at night the height on March 14 was 27 inches and there were no indications of flowering. Blossoming was inhibited indefinitely under these conditions. Seed were again sown April 6. The plants exposed to a 10-hour day beginning June 3 were showing flower buds July 27 and open blossoms on August 13. The control plants under the full day length showed no flower buds as late as September 22.

Roots of sorrel (*Rumex acetosella* L.) were transferred to the greenhouse October 12. Under natural day length aerial development was limited to abundant leaf formation, while under the artificially lengthened illumination period flowering stems were formed and pollen was falling January 19. Thereafter these latter plants continued as typical everbloomers. The plants under the short-day exposure, on the other hand, were very active in the development of underground stems, the soil of the container becoming thickly studded with this type of growth. When grown in the open bed in the greenhouse under the short-day conditions the plants produced underground stems 2 feet or more in length.

Plants of roselle (*Hibiscus sabdariffa* L.) were subjected to a 10-hour day when about 10 inches high, beginning July 20. First blossoms appeared September 11, whereas the control plants did not flower till early November.

Cuttings of smartweed (*Polyganum* sp.) were set in moist sand June 30. Under a 10-hour light exposure the plants were in blossom July 21, while the controls showed no indications of flowering on August 18. The respective heights of the plants July 28 were 14 and 9 inches.

A late fall variety of chrysanthemum was exposed to a 10-hour day, beginning May 12, and was in blossom July 28, while the control plants, of course, did not flower till late fall. Under a 13-hour exposure flowering was about 3 weeks later than under the 10-hour exposure. The 10-hour day plants flowered freely and the blossoms were normal.

A very late variety of dahlia, known as John Ehlich, was placed under a 10-hour day, beginning May 12, and the first blossom opened July 8. Under the natural day length the first blossom opened September 27.

Specimens of poinsettia (*Euphorbia heterophylla* L.), 8 to 10 inches high, exposed to 10 hours of light daily after July 9 soon flowered, and in 5 to 6 weeks the tops of the plants were gorgeously colored. (Pl. 19, A.) Similar plants exposed to 12 hours of light daily blossomed September 3, but the upper leaves reddened very slowly. The controls did not flower till November. The 12-hour plants eventually produced numerous viable

seed, whereas the 10-hour plants produced no seed. One of the control plants was transferred October 2 to the greenhouse electrically illuminated at night and under these conditions continued in the vegetative state without flowering throughout the winter.

Roots of goldenrod (*Solidago juncea* Ait.) were transferred to the greenhouse November 15, one lot receiving only natural daylight while the other lot received the electric illumination from sunset till midnight. Under the lengthened illumination period flowering began March 15. Beginning March 10, the plants in the control house were placed in a dark cabinet for a portion of each day so that they received only 7½ hours of light daily. In one instance a short, weak flowering stem developed as a result of the stimulus from the natural increase in day length before the transfer to the dark cabinet was begun. The remaining plants formed no stems but continued to form rosettes of leaves as the older leaves perished (Pl. 5, B). This type of activity, with complete suppression of stem development, persisted throughout the duration of the experiment, a period of more than 18 months.

Cuttings of hedge bindweed (*Convolvulus sepium* L.) exposed to 10 hours of light daily, beginning May 13, developed no flower buds, whereas the controls, exposed to the full day length, were in blossom June 30. Other lots of cuttings were exposed to different daylight periods, beginning June 7. The controls, as well as the plants receiving 13 hours of light daily, formed numerous flower buds and began blossoming June 28 to 30. Under a 12-hour day only three flower buds were formed on two plants, and under an 11-hour day no flower buds at all appeared.

Seed of winter and spring varieties of wheat (*Triticum vulgare* L.), rye, (*Secale cereale* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) furnished by the Office of Cereal Investigations, Bureau of Plant Industry, were planted in the electrically lighted and the control greenhouse units on November 29. The greenhouse was kept relatively warm, the temperature usually running 55° to 60° F. at night and 70° to 80° during the day. The behavior of the plants under the two conditions of illumination is summarized in part in Table II.

These results with the small grains are too limited in scope and extent to justify final conclusions, but they seem to show clearly that detailed study of these crops in their reactions to day length would give very interesting and valuable results. It appears that a fundamental distinction between the winter and spring types as such rests on the rapidity with which the latter respond to the increasing day length of spring.[4] The forcing action of the electric light on the spring types resulted in sparse fruiting, possibly because of the low intensity of the light.

[4] Since this paper was written Wanser has published in Science (WANSER, H. M. PHOTOPERIODISM OF WHEAT; A DETERMINING FACTOR IN ACCLIMATIZATION. *In* Science, n. s., v. 56, p. 313-315. 1922) an interesting account of a preliminary experiment dealing with photoperiodism in wheat, in which distinctive photoperiods are found for the jointing and the heading stages in winter wheat. The fundamental differences between winter and spring varieties of the small grains in their response to length of day brought out in the above data are confirmed by Wanser's results with wheat.

TABLE II.—*Effect of length of day on growth of winter and spring varieties of wheat, rye, oats, and barley.*

Crop.	Date of first heading.		Average height at time of heading.		Average height, May 11.		Average number of basal branches.	
	Under electric light.	Control.	Under electric light.	Control.	Under electric light.	Control.	Under electric light.	Control.
			Inches.	*Inches.*	*Inches.*	*Inches.*		
Wheat, Marquis (spring), C. I. 3641 [1]	Jan. 24	Apr. 8	28	51	36	56	8	25
Wheat, Purple Straw (winter)	Mar. 17	Mar. 23	46	41	51	52	31	13
Rye (spring), C. I. 169	Jan. 6	Mar. 29	16	25	24	56	6	30
Rye, Abruzzi (winter), C. I. 40	Jan. 24	Mar. 23	27	24	62	61	25	11
Oats, Swedish Select, (spring), C. I. 1426	Jan. 10	Mar. 28	15	51	36	72	28	19
Oats, Winter Turf, C. I. 274–20	Feb. 25	Apr. 8	49	44	61	51	33	33
Barley, Manchurian (spring), C. I. 19A625	Feb. 1	Mar. 15	36	46	40	46	16	26
Barley, (Tennessee Winter), C. I. 257	Apr. 26	Apr. 25	.....	.....	36	36	.....	.....

[1] The varietal numbers are those of the Office of Cereal Investigations, Bureau of Plant Industry.

Young plants of arrowhead (*Sagittaria latifolia* Wild), taken from a marsh, were transplanted to 3-gallon metal buckets with perforated bottom and containing garden soil maintained at approximately optimum moisture content for such plants as soybeans. Similar lots of plants also were transplanted to 3-gallon earthen jars containing 6 inches of soil covered to a depth of 4 to 5 inches with water. Under a 10-hour day, beginning May 3, two plants in a metal bucket flowered June 14 and 17, respectively, and three individuals in an earthen jar flowered June 28, July 5, and July 9, respectively. Two controls in each of two earthen jars, exposed to the full day length, flowered August 19, 22, 24, and 27, respectively. The plants in the metal bucket were restored to the full daylight period June 17, and one of these flowered the second time on August 22. In a second series, transplanted May 15, three plants in each of two earthen jars exposed to 10 hours of light daily, beginning May 15, began flowering June 11 and 14, respectively. Under 10-hour day, beginning June 13, four plants in an earthen jar flowered June 27, 28, 29, and 30, respectively, while under a 12-hour day one of three plants flowered on June 30 and the other two on July 2. Under a 10-hour day, beginning June 23, two plants in a metal bucket flowered July 5. The controls of the second series, transplanted May 15 to an earthen jar and to a metal bucket, had not flowered September 20.

Very young plants of jewelweed (*Impatiens biflora* Walt) were transferred from the swamp to 3-gallon metal buckets April 12, five plants being set in each bucket. Under a 10-hour day flowering began June 7. The controls began flowering July 6.

Morning-glory (*Ipomoea hederacea* Jacq.) was planted June 8 and had germinated June 11. Under a 10-hour day flower buds appeared July 2 and the first open blossoms were in evidence July 10 (Pl. 2, B).

The controls showed flower buds August 25 and open blossoms September 9. The 10-hour light exposure produced a decidedly darker shade of green in the foliage leaves than did the full summer day length. These short-day plants continued to grow slowly through the flowering period and were still green September 23.

Seeds of Brazilian morning-glory (*Ipomoea setosa* L.) were planted June 8, and the seedlings had emerged from the ground on June 11. Exposed to 10 hours of light daily, the plants were showing flower buds July 5 and the first blossoms opened July 19. On the controls flower buds could be seen August 25 and the first blossoms opened September 9.

The dwarf morning-glory, *Convolvulus tricolor* L., was planted June 8 and had germinated June 11. Under a 10-hour day the plants were unable to flower but remained green and continued to grow vigorously. The controls under the natural length of day were showing flower buds August 1 and first open blossoms August 11. After a period of flowering through August these plants reverted to the purely vegetative phase of activity after the first week in September, assuming a condition comparable with that of the plants exposed to a 10-hour day.

Moonvine (*Calonyction aculeatum* (L.) House) planted June 8 had germinated June 11. With 10 hours of light daily flower buds appeared July 15 and open blossoms August 5. On the controls flower buds could be seen July 23 and open blossoms August 16.

Seeds of cypress vine (*Quamoclit pennata* (Desr.) Voigt) were planted June 8 and appeared above ground June 11. Under 10 hours of light daily, flower buds were showing June 22 and the first blossoms were open July 5. On the controls the first open blossoms appeared August 3. The short-day plants grew more rapidly and were darker in color than the controls exposed to the full day lenght.

LONG-DAY AND SHORT-DAY PLANTS AND THE CRITICAL LENGTH OF DAY FOR FLOWERING

As pointed out in the earlier paper and further illustrated in the preceding data, the plants studied tend to arrange themselves into two groups. One of these groups consists of species that are caused to flower by the action of short days while the other includes those species that are forced into flowering through the action of long days. For convenience the first named group are spoken of as short-day plants while the second group are designated as long-day plants. At first sight it would seem that these two groups of plants are diametrically opposed in their response to length of day, but detailed study of the two groups indicates that the difference is one of degree rather than of kind. In fact, classification into the two groups is more or less arbitrary. In the case of such plants as Cosmos and Bidens, flowering is inhibited by a daily light exposure much in excess of 12 hours' duration, while vegetative development promptly gives way to flowering when the light period is reduced to 12 hours or less. These plants will flower even when receiving only a few hours of light daily; in other words, it is not possible to reduce the light duration sufficiently to prevent flowering without killing the plants. On the other hand, Solidago and Hibiscus are readily prevented from flowering by reducing the light period to something less than 12 hours, although under these conditions they may continue to live and may even continue development in certain directions. These plants are not inhibited from flowering by increasing the light duration beyond the normal.

The important point is that the inhibition of flowering in the first case by a long light period is not of the same sort as the inhibiting effect of a short light period in the second case; that is, the inhibition in the two cases results in different alternative forms of vegetative activity, as will be shown in later paragraphs. There are, moreover, plants which may be said to occupy an intermediate position in that it is possible to have a day length too long as well as one too short to induce flowering. *Mikania scandens* L., briefly discussed in our former paper (7), is an example of this type. In the wild state Mikania regularly flowers in late July and through August, and if kept in the greenhouse through the winter it also usually flowers very sparsely in the spring. Seedlings from a planting made December 10 were unable to flower under the influence of the lengthened daily light period in the electrically illuminated greenhouse, while similar plants in the control greenhouse flowered at the usual time in August of the following summer. On the other hand, this species was unable to flower through the summer or fall under day lengths of 5, 10, and 13 hours' duration. The Biloxi variety of soybeans (*Soja max* (L.) Piper), discussed in detail in the former paper (7), occupies a position slightly below the intermediate position of Mikania in response to day length. In this case flowering is readily inhibited by a day length in excess of 13 hours. On the other hand, with very short day lengths only a very few cleistogamous flowers and fewer seed are developed but the reproductive phases apparently can not be entirely suppressed.

Thus, there are species occupying various positions within the annual range of day length with respect to the initiation of reproductive activities, and there is no definite line of division between long-day plants and short-day plants. An illumination period on one side of the critical duration for flowering promotes certain forms of vegetative activity, while a light period lying on the other side of the critical promotes other forms of vegetative activity, as will be shown under the discussion of apogeotropism (p. 886).

Not only do plants differ markedly as to the particular length of day most favorable for flowering but they also differ widely as to the narrowness of the range in day length which will permit of flowering. Experiments with Mikania, referred to above, indicate that under ordinary conditions this plant will readily flower only under a day length ranging but little beyond an hour on either side of 14½ hours, which is approximately the optimum for flowering. Buckwheat flowers readily under a daily illumination period ranging at least from 5 hours up to 18 or 20 hours and probably even under continuous illumination, notwithstanding the fact that the extent of vegetative development and life duration are profoundly affected by this range in the length of the light period. While there is a certain degree of antagonism or incompatibility between the vegetative and reproductive phases of activity, the two obviously are not necessarily affected to the same degree by a given change in duration of the light period. *Viola papilionacea* Pursh will flower under all lengths of day met with in temperate regions except for a short period in midwinter. In the broadest sense the plant will flower continuously for about 10 months of the year, a conspicuous example of everblooming. During the summer months, however, only the cleistogamous type of flower is seen while in spring and fall only the showy blue, chasmogamous blossoms develop. By maintaining a light period equivalent to the long summer days the cleistogamous flowering continues indefinitely and, similarly, a considerably shorter light period will maintain the

open type of flowering indefinitely, thus illustrating a narrower type of everblooming (*8*). In this case the whole effect of the range in length of day from February to the summer solstice is merely to change the type of blossom (involving change in relative fertility).

Thus, beginning with the equatorial length of day of 12 hours as the standard, it may be said that a group of plants normally will flower under any range downward to less than 6 hours, while another group will flower under any range upward to at least 18 hours and probably up to continuous illumination. Other plants will flower only within a comparatively narrow range on either side of the 12-hour standard. Still others are capable of flowering throughout these ranges, their response being quantitative rather than qualitative in character.

BALANCE BETWEEN FLOWERING AND VEGETATIVE ACTIVITY

Within the range of duration of light exposures which will admit of alternative forms of expression there are in most cases certain exposures favorable only to sexual reproduction and other exposures favorable only to vegetative activity. That is, there are more or less definite optima in light exposures for the two alternative forms of expression. The question arises as to the response of the plant to light exposures intermediate between those specifically favorable to the two types of activity. In reality the effects of these intermediate exposures are important and are both quantitative and qualitative in character. There are two aspects of the problem. Under artificial control, fixed day lengths of intermediate duration may be studied, whereas, in nature, there is a graduated change from one optimum toward the other and of course the change may be in either direction.

One of the effects of an intermediate light exposure is a tendency toward the condition of everflowering in which the plant divides its activity between the vegetative and the reproductive phases of development. As the day length approaches the optimum for flowering, the vegetative phase of activity is reduced more or less and vice versa. Thus, the Yellow Dent variety of corn grown in summer in the greenhouse, with electric light at night to give an illumination period of 18 hours, produced larger, taller, and longer-lived stalks while the ears were longer, with longer stems, but were poorly filled as compared with the controls exposed to the normal summer day length. Again, under a 10-hour day Biloxi soybeans gave per plant a dry weight of 10.9 gm. of stalk and 36.6 gm. of seeds, a ratio of 1 to 3.3. The corresponding values under a 13-hour day were 65.8 gm. of stalk and 95.3 gm. of seeds, a ratio of 1 to 1.4. Under the 10-hour day growth ceased as soon as flowering began, but under the 13-hour day there was a tendency toward everbearing, for growth and fruiting proceeded simultaneously. A considerable volume of data which need not be considered in detail here has been obtained with soybeans grown under artificially regulated day lengths and planted in the field at stated intervals during the season, all of which further exemplify this principle of the influence of intermediate day lengths on the quantitative relation between vegetative and reproductive activities.

TABLE III.—*Effect of length of the daily light period on the size of seed in different varieties of soybeans*

Length of daily light exposure.	Average weight of 500 seeds.			
	Mandarin.	Peking.	Tokyo.	Biloxi.
	Gm.	*Gm.*	*Gm.*	*Gm.*
Germinated May 17:				
Full daylight throughout, 15 to 11½ hours	100.0	43.2	110.4	Did not mature.
7 hours throughout	67.6	48.4	130.6	131.2
5 hours till 1 week after flowering; full daylight thereafter	85.8	36.0	60.4	88.4
Full daylight till flowering; 7 hours thereafter	.....	49.2	.....	.....
Full daylight till flowering; 5 hours thereafter	.....	37.0	105.0	.....
Germinated June 15:				
Full daylight throughout, 15 to 11½ hours	93.8	37.4	99.8	Did not mature.
12 hours throughout	89.4	62.8	125.8	129.8
Darkness 10 a. m. to 2 p. m. (and at night) throughout	.....	30.0	.....	.....

It has been found, also, that the size of the individual seed is influenced by the relation of the prevailing light period to the respective optimal period for vegetative and reproductive development. The data presented in Table III throw light on this question. It is seen that in the early variety of soybeans, Mandarin, which fruits readily in the longest days of summer, shortening the light period to 12 hours or less reduced the size of the seed and restoring the plant to the full summer daylight period after flowering had occurred tended to offset the unfavorable influence of the previous exposure to 5 hours of light daily. With the later varieties, requiring shorter days for flowering, the results are quite different. For these a 12-hour exposure gives decidedly larger seeds than the full day length of summer and for the Tokyo and Biloxi a 7-hour day gives fully as large seed as a 12-hour day. A 5-hour light period till flowering, followed by exposure to the full day length, reduced the size of the seed markedly in all except the early variety. A midday period of darkness acted unfavorably. All these data harmonize with previous results on the action of regulated day lengths in initiating or inhibiting flowering and fruiting in these varieties of soybeans.

An intermediate light exposure tends to delay the time of flowering, and as the light period is advanced toward the optimum for vegetative development flowering is more and more delayed, the tendency being toward more or less indefinite inhibition. Biloxi soybeans which germinated June 1 began flowering June 27 under a 10-hour day, while the first blossoms appeared July 13, 16 days later, under a 13-hour day. Other plantings gave very similar results. It has previously been shown that a day length of 14 hours or more delays flowering indefinitely in the Biloxi. The Peking variety planted on June 18 began flowering July 14 under a 13-hour day as well as under a 10-hour day for the reason that the 13-hour day length does not materially exceed the optimum for flowering in this variety. It should be noted, however, that development and ripening of the seed of the Peking are decidedly hastened by

the 10-hour day as compared with the 13-hour day. By referring to Table I it will be seen that a 13-hour day delayed flowering in varying degrees in different varieties of sorghum as compared with a 10-hour day. As previously noted, flowering in chrysanthemum and poinsettia, also, was delayed by a 13-hour exposure as compared with a 10-hour exposure. As a rather extreme case reference is made to the behavior of wild sunflower as described on page 875. In this instance the unfolding of the flower buds was delayed for several months by the long daily light exposure.

As the light period is shifted away from the flowering optimum and toward the optimum for vegetative development one of the effects is cleistogamy. Thus, the sparse flowering of Mikania in spring, previously referred to, when the increasing day length is moving toward the optimum for vegetative activity, is usually associated with a cleistogamous type of blossom. The cleistogamous flower of the violet during the long summer days is to be regarded as a step away from the open, blue, colored blossom of spring and toward the purely vegetative form of development. These results are in line with observations of Bouché (4) on certain species, reported in 1875.

CHANGE FROM THE REPRODUCTIVE TO THE PURELY VEGETATIVE FORM OF ACTIVITY

It is of interest to inquire further into the effect of changing the plant from the optimal light exposure for sexual reproduction to one approximating the optimal for vegetative activity. If made in the earliest stages of development of the plant the change in light exposure would have little or no qualitative effect, while if made only after senescence has reached an advanced stage the only action of importance would necessarily involve rejuvenescence. This latter condition will be considered in later paragraphs.

Plantings of *Cosmos bipinnata* Cav. in 12-quart galvanized iron buckets germinated July 5, each bucket containing about a dozen seedlings. At the outset all plants were exposed to a 10-hour day, a light period which quickly forces flowering. Beginning July 6, one bucket of seedlings was transferred on each alternate day from the 10-hour day to the full daylight exposure of summer at Washington, D. C. All control plants remaining under the 10-hour day were showing flower buds on July 26. All plants transferred to the long-day conditions on or after July 18 likewise were showing flower buds on July 26, while the plants transferred during the period July 6 to 16, were showing no flower buds as late as September 2. It is evident that an exposure to a 10-hour day for not less than 10 days is necessary to bring the flower buds into evidence. The dates of first open blossoms ranged from August 9 for the plants remaining under the 10-hour day till July 26, to August 21 for those transferred to the long-day conditions on July 18. A notable feature was that of the numerous flower buds formed on the plants which were removed from the 10-hour light exposure on July 18 and July 20 only a few were able to open, the others being permanently suppressed, a result which has often been observed in cosmos and other plants. The foreshortening of the primary axis, with resultant crowding together of nodes, associated with the formation of the terminal flower bud, is followed by considerable swelling of the nodal tissues immediately beneath the bud as a result of the stimulus from the longer day. New

vegetative shoots encircling the flower buds soon begin to elongate, and the latter apparently is deprived of the necessary nutrition for development. It should be pointed out that in this experiment the formation of flower buds in all cases was associated with marked branching at the tops of the plants, while branching did not occur in those plants in which flower-bud formation was inhibited by the lengthened light exposure.

A similar experiment was undertaken with Biloxi soybeans. The seed, sown in 12-quart buckets, germinated July 2, and at the outset all seedlings were exposed to a 10-hour day. Beginning July 6 one lot of seedlings was transferred each succeeding day to permanent outdoor conditions, the last lot being transferred July 25. The results are shown in Table IV. The response to the change in the light period is similar to that of Cosmos. Here, again, 10 days' exposure to short-day conditions was the minimum for inducing flower formation. All plants thus responding also flowered again at the normal time for the variety in early fall as a result of the natural shortening of the day. It will be observed that although flowering was successfully accomplished where the soy beans were exposed to the 10-hour day for 10 to 20 days no fruits were developed, a fact which is regarded as important. Although the blossoms appeared to be normal it seems that under the influence of the long-day exposure even the stimulus of fertilization was not sufficient to permit development of the seed. Beginning with an exposure to the 10-hour daily light period for 11 days the fruits in increasing numbers were successfully developed.

TABLE IV.—*Effect of transferring Biloxi soybeans at intervals of one day from a 10-hour daily light exposure to the full daylight period of summer*

Date transferred to full daylight.	Date of opening of first blossom.	Date of second (normal) blossoming period.	Average height of plants.	Seed formation.
			Inches.	
July 6	Sept. 13	.....	42	No pods formed.
7	...do.....	.....	42	Do.
8	Sept. 14	.....	42	Do.
9	Sept. 13	.....	42	Do.
10	...do.....	.....	42	Do.
11	...do.....	.....	42	Do.
12	Aug. 9	Sept 15	42	No pods formed from first blossoms.
13	...do.....	Sept. 14	42	Do.
14	...do.....	...do.....	42	Do.
15	July 31	...do.....	40	Do.
16	...do.....	...do.....	40	Do.
17	...do.....	...do.....	40	Do.
18	...do.....	...do.....	40	Do.
19	July 27	...do.....	39	Do.
20	July 26	...do.....	38	Do.
21	...do.....	...do.....	36	Do.
22	...do.....	...do.....	36	Do.
23	...do.....	...do.....	35	A few pods formed from first blossoms.
24	...do.....	...do.....	32	Numerous pods formed from first blossoms.
25	...do.....	...do.....	28	Do.

SPRING FLOWERING AND FALL FLOWERING

The facts brought out in the preceding discussion of the relation between long-day and short-day plants and the balance between the vegetative and the reproductive types of activity throw light on the relationships existing between spring-flowering and fall-flowering plants. As is well known, many plants normally flower only during the spring, while many others regularly flower only in the fall. There are a number of plants, however, which flower in both spring and fall, although usually there is a marked difference in the extent of the flowering during the two seasons. Finally, there is a large group of plants which blossom in midsummer and a smaller group which under favorable conditions flower in winter. It is well known that in a very large number of perennials the flower buds are organized during the summer or early fall of the year preceding that in which the blossoms finally open, so that, in considering the effects of light duration, it is necessary to avoid confusing the action on the laying down of primordia and that on the final unfolding of the blossom.

Broadly speaking, in cool temperate regions short-day plants will flower chiefly in the fall rather than in the spring because of the lag in temperature rise in spring as compared with the lengthening of the day. In other words, in spring the day length is likely to become too long for flowering of short-day plants before the temperature has risen sufficiently to permit plants to become active. This is true more particularly of the annuals and those herbaceous perennials which require considerable vegetative development as an antecedent to flowering. That plants of these types which regularly flower in the fall will actually flower in the spring when the obstacle of low temperature is removed has been demonstrated in a number of cases. As a specific illustration Peking soybeans germinating in the greenhouse March 31 showed first open blossoms on May 12, or 42 days after appearance above ground, while a second lot germinating April 13, just two weeks later than the first planting, did not show open blossoms till July 16, 90 days after germination. Plantings of the Biloxi soybeans made on the same dates failed to flower till September, since the day length was already above the critical for flowering in this variety. The Biloxi quickly flowers, however, if planted in winter or early spring in the greenhouse. Indirectly, the many experiments already described in which various plants have been forced into flowering out of season by shortening the light period or inhibited from flowering in the normal season by increasing the light period furnish proof of this relationship of spring and fall flowering in the short-day plants. As the critical light period for flowering becomes longer the chances for spring flowering of the species are increased till finally spring flowering merges with summer flowering in those species which are intermediate between the more typical short-day and long-day plants. Again, sparse flowering in spring is to be looked for in those of the short-day plants which are able to flower at all, for the reason that the change in day length is toward the optimum for vegetative activity and away from the optimum for flowering. It is under these conditions, also, that various modifications and abnormalities in flowering and fruiting, quantitative as well as qualitative, are most likely to occur. On the other hand, the short-day plants as a whole will have their energies thrown into flowering and fruiting more or less quantita-

tively, as it were, through the change in day length in the fall when the change is away from the optimum for vegetative activity and toward the optimum for sexual reproduction. One importamt qualification must be made to this principle, namely, that it holds good only so long as the day length does not become too short for the flowering and fruiting processes.

The long-day plants, on the other hand, through late spring and early summer are subjected to a changing day length which is approaching the optimal for sexual reproduction, hence it is to be expected that these plants would flower and fruit abundantly during this period. A large proportion of spring flowering plants consists of woody and herbaceous perennials in which the flower primordia are organized during the summer preceding the spring in which the blossoms unfold. In this case it seems probable that the optimal day length for flower development following the summer solstice passes by before the embryonic flower buds have made much progress in their development and the direction of the change in day length through late summer and the fall is away from this optimum for flowering and fruiting and toward or through the optimum for vegetative activity. Under these conditions further development of the flower buds would be attended with difficulty so that their growth would be slow. If the onset of cold weather is delayed, some species may flower in the fall instead of in the normal spring season. In some, and probably in most instances, however, flowering would be inhibited by the short days of late fall and winter. On the other hand, the cold weather of winter may exercise a distinctly helpful influence on the completion of the flowering process by establishing a favorable balance of income over outgo. Consequently, the earliness of flowering in the spring will depend largely on how soon the temperature rises to the point where development can actively proceed. The necessary internal conditions for flowering having been established, it remains for rise in temperature in spring to speed up the unfolding of the blossoms. Nevertheless, the increasing length of the day in the spring undoubtedly remains a factor, particularly in those species which do not unfold their blossoms till spring is well advanced. This is seen, for example, in the case of the beet (p. 888).

APOGEOTROPISM, OR INCREASE IN STATURE

Apogeotropism or increase in stature is a response of great importance in photoperiodism; and, in fact, the experimental data thus far secured as a whole seem to indicate that in a sense this is the fundamental phenomenon underlying the various other responses, including flowering and fruiting, which are discussed in this paper. It appears that, in general, there is an optimal length of day for apogeotropic growth and any change to suboptimal conditions will result in checking the apogeotropic type of vegetative development, to be followed by various other forms of expression. With sufficient departure from the optimal light period the primary axis may be completely suppressed and aerial growth practically confined to leaf development, as typically shown in the leaf rosette of stemless plants. Another typical form of expression resulting from a rather extreme suboptimal light period is the prostrate or creeping habit of growth, stem elongation taking place without increase in stature. As the day length is advanced toward the optimum the rate of vertical elongation, as well as the final stature attained, are increased.

This relationship seems to hold whether or not flowering is involved and applies also to branches and leaf stems as well as to the primary axis. The seasonal length of day may be either too long or too short for maximal apogeotropic response.

It is probable that the first important result of departure from the optimal day length for apogeotropism is a foreshortening of the stem which results in, or at least is accompanied by, transformation of the terminal portion into an inflorescence. With a more marked departure from this optimum, such as would completely eliminate apogeotropic stem elongation, flowering gives way to leaf development. In other words, the foreshortening is pushed to the point where the terminal flower bud is replaced by the leaf rosette. This, at least, expresses the result morphologically even if it should have no physiological basis. As has been previously pointed out, many short-day plants of the type of Cosmos and Bidens continue for a more or less indefinite period vertical elongation of the axis under the influence of long days. As the day length is reduced to suboptimum, elongation of the axis is checked and flowering quickly follows. In these plants stem elongation can scarcely be completely eliminated by reducing the day length, and likewise flowering can not be readily inhibited by this means. In many long-day plants, on the other hand, apogeotropism may not be pushed so far as to inhibit the foreshortening and flowering, but by reducing the day length initial elongation of the axis is readily prevented. It is probable, moreover, that some of the woody perennials will be found to occupy an intermediate position in which more or less indefinite apogeotropic growth is induced by the optimal light period, on the one hand, and such growth completely inhibited, on the other hand, by suitable change of the light period to suboptimum.

A few examples illustrative of the above-mentioned responses will be of interest. In Comos, as stated on page 875, the axis reached a height of 15 feet under the influence of a long daily light exposure, and then flowering occurred only because of a decrease in the light period, whereas the final height attained under short-day conditions throughout was only 30 inches. Bidens, which behaves like Cosmos under long-day conditions, may attain a height of only 2 inches when exposed to a short-day length, the foreshortening of the axis being followed by flowering. It was pointed out, also, that while buckwheat flowers under all day lengths employed, under the longer light periods flowering is accompanied by marked increase in stature, the final height in this case being more or less indeterminate. Similarly, under an increase in duration of the light period from the normal day length of winter to about 18 hours, which was without effect on the time of flowering in Connecticut Broadleaf tobacco (*Nicotiana tabacum* L.), the height was increased by 50 per cent and the number of leaves was doubled. In the violet the leaf stalk is greatly lengthened by increase in the length of day. Goldenrod, as described on page 877 and illustrated in Plate 5, B, furnishes a striking example of inhibition of stem elongation by a reduced day length.

Summer radish (*Raphanus sativus* L.) when exposed to a daily light period of 7 hours is unable to develop a flowering stem, but under these conditions the leaves reach an unusually large size and the root continues to enlarge. Two plants which had received 7 hours of light daily during the preceding summer and fall were exposed to the natural day length of winter in the greenhouse till February 1. At that time it was seen that

in one of the individuals the increasing length of day had initiated development of a flowering stem. These plants were then restored to the 7-hour day length. In the plant which had begun the development of a stem, elongation of this stem was soon checked, the final height reached being only 24 inches (Pl. 3, A). This is in spite of the fact that the root had attained a diameter of nearly 5 inches. Final features of development in this interesting plant, as shown in Plate 3, B, will be considered under the subject of branching (p. 899). In spite of the large quantity of carbohydrate material stored in the root the height of the stem was sharply limited by the duration of the light period. The beet (*Beta vulgaris* L.) differs from the radish in that it usually behaves as a biennial and Klebs (*11, p. 292*) showed several years ago that this plant may be unable to flower the second season if kept in a warm greenhouse through the winter. Large roots which had been stored in an outdoor pit during the winter were set in soil and one lot placed under a 10-hour day on April 1. The control plants under natural day length flowered late in June as indicated in Plate *8*, B. Under the 10-hour light period elongation of the stems soon ceased and the apical buds developed into leaf rosettes. The 10-hour day was so far below the optimum for apogeotropic development that foreshortening apparently was too abrupt to permit of flowering. The final result of the short-day conditions was that aerial "beets" (thickened stems) were formed at the bases of the leaf rosettes. Referring back to the behavior of tropical sorghums (Table I), it may be noted that a day length of 13 hours approximates the optimum for apogeotropic response in some of these varieties, as for example in S. P. I. 43626. After the flowering stage had been reached the final heights under the 13-hour day were decidedly in excess of those under the full day length or the 10-hour day. The full day length evidently is excessive, while the 10-hour day is less favorable than the 13-hour day.

Preliminary observations on the growth of the apple (*Malus sylvestris* Mill) in contrast with that of *Acer negundo* L., for example, under different day lengths are of special interest. As will be seen by reference to Plate 4, B, seedlings of the latter are scarcely able to grow at all under a 10-hour day. In marked contrast, the apple grows more rapidly under a 10-hour day than under the full day length of midsummer. Young seedlings under the two light exposures are shown in Plate 4, A. Two specimens of standard Baldwin grafts and one of Baldwin grafted on a Paradise dwarf stock which were transplanted to large tubs in April and exposed to 12 hours of light daily soon began a rapid rate of growth which was maintained throughout the summer (Pl. 5, A). Of the three corresponding individuals similarly transplanted but exposed to the full daylight of late spring and summer, only the graft on Paradise stock survived, and up to August this grew much more slowly than the trees exposed to a 12-hour day. During August, however, it was seen that there was marked acceleration in the rate of growth of the control, continuing through early fall which, of course, is in line with the normal behavior of the apple. To arrive at the full significance of these results will require further study, but they seem to indicate that the long days of midsummer occurring in high latitudes are distinctly suboptimal for apogeotropic vegetative development in the case of the apple and therefore probably conducive to the initiation of flower-bud formation. As the tropics are approached the shorter length of the summer day would favor vegetative activity at the expense of flower-bud formation.

Similarly, since the general change from long to shorter days with the advent of late summer would favor vegetative activity, the growth and development of flower buds would be correspondingly retarded and, finally, cold weather, also, would operate to slow down both types of activity. Thus, the tendency would be toward sparse fruiting and rapid vegetative development with advance from high latitudes toward the equator; the shortening days of late summer would tend to delay final flowering till the following spring, particularly in high latitudes.

FORMATION OF BULBS, TUBERS, AND THICKENED ROOTS

Tuberization is one of the outstanding features of photoperiodism This fact is not so surprising, perhaps, in view of the significane of stem elongation as a fundamental factor in various responses of the plant to change in the daylight period. As is well known, tubers and bulbs represent extreme conditions of stem shortening accompanied by marked increase in diameter. Using the term tuberization in its broadest sense to include the formation of thickened roots as well as the more typical tubers, bulbs, and corms, the general feature of carbohydrate accumulation may be regarded as being common to all these storage organs. This accumulation obviously indicates an excess of carbohydrate over current consumption, but the important point to be emphasized in the present discussion is that the surplus of carbohydrate is not due to increased photosynthetic activity but rather inability on the part of the plant to utilize the carbohydrate, whether it be present in relatively large or small quantity.

As to the locus of the tuber or other storage organ it appears that the presence of previously formed buds or the ability to organize such structures is an important factor. Environmental conditions also play a part in determining the position of storage organs as well as in their formation. The work of Vöchting on this subject is of special importance. This investigator (*22*) showed that shoots of the potato (*Solanum tuberosum* L.) set in the soil in such manner that the underground portion consists only of an internodal section readily forms aerial tubers for the reason that the underground portion is incapable of developing adventitious buds. It was shown, also, that by suitably darkening the apex of the primary shoot on a branch the growing point is converted into a tuber and, consequently, it was concluded that darkeness favors tuber formation.

In later experiments with tubers of the Marjolin variety of potato taken from storage after having sprouted (*23*) Vöchting found that when germinated in darkness and with abundant moisture in soil and air roots and foliage shoots quickly developed but no new tubers were formed if the temperature was maintained at 25° to 27° C. With a temperature of 5° to 7°, on the other hand, no foliage shoots were formed, but instead numerous new tubers developed at the ends of old sprouts and new stolons and on the mother tuber. In other words, the growing points were tuberized. By transfer from the higher to the lower temperature the foliage shoots assumed horizontal direction of growth and formation of tubers began. Experiments with low partial pressures of oxygen indicated that decreased respiratory activity is not responsible for the low-temperature effect. It is important to observe, however, that germination in sand free from moisture gave no foliage shoots but only short roots and stolons bearing numerous tubers, regardless of whether the tem-

perature was high or low. By reducing the moisture supply after a foliage shoot some 10 to 12 cm. high had developed as a result of an abundant supply of moisture, further upward growth was stopped and numerous small aerial tubers soon formed. The picture thus presented (*23*, *Table IV*, *p. 114*) strongly calls to mind the general contour of a miniature shrub, defoliated but bearing numerous fruits. With moist soil but dry atmosphere the shoots which developed soon assumed a horizontal direction and numerous basal branches appeared. In germination tests in diffuse light a moist soil gave relatively large leaves and long internodes in the presence of moist air and short, thick internodes, mere scales rather than true leaves, and basal stolons in the presence of dry air. Tubers set upright in plates, without use of any soil, showed decreased elongation of the apical shoot with increased light intensity. Under these conditions light inhibited root formation.

In contrast with Vöchting's work on tuber formation, Bernard, in a lengthy article (*1*) dealing largely with the life history of the orchids, reached the conclusion that in many species a period of active differentiation is followed by a definite period of tuberization, while in other species tuberization begins in the earliest stages of development. In all cases Bernard found a close correlation between tuberization and infection with certain endophytic fungi and concluded that these parasites are the cause of tuber formation. This fungus theory of tuberization was extended to include the potato. It had been previously shown by Laurent (*12*), however, that shoots from potato tubers, which had been grown in darkness and were free from starch, developed tubercles in darkness when placed in saccharose solutions of proper concentration (10 to 20 per cent). Having subsequently confirmed these results Bernard (*2*) was forced to modify his views as to infection by fungi being the sole cause of tuberization. Molliard (*16*) showed that onion (*Allium cepa* L.) grown in a sterilized nutrient solution containing glucose is capable of forming a bulb and later (*17*) showed, also, that radish and certain other species form a starchy, thickened root under similar conditions. Thus, while it may be possible that in special cases tuberization is due to the invasion of fungi, it seems certain that parasitism or symbiosis is not the usual cause of the formation of tubers and other storage organs. Magrou, in a recent extended discussion (*15*), however, seeks to establish the importance of Bernard's fungal theory of tuberization.

"Sets" of the common Silverskin variety of onion were grown under different day lengths, 15 to 20 individuals being grown in each lot. In the first test the sets were planted in the greenhouse May 19. One lot was exposed to the natural day length while the second lot received in addition electric illumination of 3 to 5 foot-candles from sunset till midnight in the manner previously described. The summer temperature in the greenhouse was high, averaging 10° to 20° F. above the outside air temperature. The behavior of the plants both with and without artificial illumination was much the same as that of the outdoor controls except that the size of both tops and bulb was considerably reduced, as shown in Plate 9, A. The controls exposed to the full day length of summer developed in the usual manner. First blossoms opened July 14, large bulbs were formed, the tops died in due course, and there was no splitting into new individuals. The resting bulbs resumed vegetative development in September. A series exposed to 13 hours of light daily out of doors did not flower and the tops remained green throughout the summer.

The original bulbs in most instances gave rise to several new individuals, each of which eventually formed bulbs of considerable size. Under the full-day length only single stalks were formed, while under a 10-hour day each unit gave rise to several stalks. Under the 10-hour day there was permanent failure to form bulbs although the plants split into two or more new individuals. The appearance was that of spring onions, and the tops remained green for an indefinite period. Though the plants have remained green for more than 12 months there has never been any indication of flowering or bulb formation. With only 5 hours of light daily, weak shoots of pale green color developed and no bulbs were formed. A surprising fact is that flower buds were formed, though these were not able to open. This behavior possibly indicates that for plants of this type, having an intermediate length of day as optimum for vegetative growth, there may be two-day lengths favorable for flowering. The plants under the 5-hour day were short-lived. The effects of the exposures on formation of tops and bulbs are well shown in Plate 9, A. These results indicate that in the onion bulb formation as well as flowering and the resultant rest period are induced by the long days of summer while a shorter day length favors a period of vegetative activity of indefinite duration, thus eliminating bulb formation and the rest period which normally follows. The shorter day length does not interfere with the splitting into new units. The onion affords an instance in which the optimal day length for vegetative growth is relatively short while a long day results in dormancy following the development of a resting organ representing extreme shortening of the primary axis. Referring to Plate 9, A, it is interesting to note that the high temperature of the greenhouse greatly reduced the size of the plant as a whole but did not alter the general morphology. The addition of the electric light of very low intensity shows a slight attenuating effect, the plants being somewhat taller than those exposed only to natural daylight.

Tubers of the McCormick, a late variety of potato, were cut in the usual fashion and planted in the greenhouse May 21. One lot of the potatoes was exposed to natural daylight only while a second lot received, in addition, artificial illumination of 3 to 5 foot-candles from sunset till midnight in the manner previously described. As previously stated, the temperature in the greenhouse averaged 10° to 20° F. above the outside air temperature. In the house in which no artificial illumination was used the first blossoms opened July 3 when the plants were about 27 inches high. When harvested November 1 the average weight per plant of green tops and tubers combined was 1,127 gm. and that of the tubers alone was 340 gm. The average height of the plants was about 6 feet. The tubers were of small size. In the electrically lighted house flower buds formed, but none of them were able to unfold. When harvested November 1 the average height of the plants was 8½ feet and the average green weight was 1,528 gm. In this case no tubers whatever were formed, so that under the influence of the increased illumination period both sexual and vegetative forms of reproduction were suppressed. All energies of the plant were directed toward vegetative development. Tubers are resting organs, and the longer light period did not permit of slowing down in vegetative activity or any tendency toward dormancy. This is illustrated by the fact that an underground bud which otherwise might have given rise to a tuber germinated and, in effect, developed an offset, the usual rest period of the tuber being eliminated (Pl. 8, A). Similar plantings of the McCormick variety also were made in boxes on

May 21 and were exposed to regulated day lengths by use of the dark houses. The exposures were 5, 10, 13 hours, and the full daylight period of the season. The plants were harvested August 28. The results of the test are summarized in part in Table V. The conditions in general and the soil in particular were unfavorable for the best development of tubers, but for comparative purposes the results are of considerable interest.

TABLE V.—*Results with McCormick variety of potato exposed to different daily light periods*

Duration of daily light period.	Date of first blossom.	Average height when harvested.	Average weight of tubers.	Average weight of tops, air-dried.	Ratio of tubers to air-dried tops.	Condition of tops when harvested.
		Inches.	*Gm.*	*Gm.*		
5 hours	July 2	20	80	2.8	12.1	All dead.
10 hours	...do	12	320	1.9	168.1	Mostly dead.
13 hours	...do	25	664	30.9	22.1	Green.
Full daylight period	July 23	32	471	32.0	15.1	Do.

With exposures of 5 and 10 hours of light daily the blossoms unfolded but no seed balls were formed. Taking the two experiments together it appears that a very long daylight period tends to direct the activities of the plant toward vegetative development to the exclusion of other alternative forms of expression. With a somewhat shorter daylight period the tendency is toward successful sexual reproduction and moderate tuber formation. With further shortening of the daylight period seed development again fails and there is a tendency toward tuber formation to the exclusion, as far as may be, of other alternative types of expression. Finally, with an extremely short daylight period it is seen that there is some tendency toward return to exclusive vegetative development. With a light duration of 10 hours or thereabouts there is a condition of intense tuber formation; in other words, there is an optimal light duration for tuberization as well as for flowering and fruiting and for purely vegetative development. Since one of the limiting factors in tuber formation is the quantity of photosynthetic material formed, the actual or absolute weight of tubers is greatest under a somewhat longer daylight period, namely 13 hours. Under natural conditions, however, the seasonal range in day length becomes an important factor; and it may well be that in northern latitudes the long days of summer leading to extensive foliage development, followed in autumn by a correspondingly precipitate decline in day length, constitute a very favorable situation for tuber development. It may be added that nothing in this discussion should be construed as minimizing the significance of relatively low temperatures in tuber development, particularly with respect to size of tuber.

Tubers of ground nut (*Apios tuberosa* Moench) planted in boxes in the greenhouse early in March germinated April 6. Beginning April 20, one lot was exposed to 10 hours of light daily while a second lot was exposed to the full seasonal day length. Flower buds were showing on both lots when the light treatment began. On the control plants exposed to the full-day length the first open blossoms appeared June 1, and these plants were in full bloom till late in August. Under 10 hours of light

daily only 1 or 2 blossoms opened on each plant, the remaining flower buds being suppressed. The leaves assumed a dark green color, growth of the vines ceased and above ground the general appearance was suggestive of a sort of dormancy. In September both lots of plants died back to the ground level. The underground portions were harvested October 28. The control plants had produced a network of small tubers throughout the soil of the boxes, but the mother tubers had enlarged but little and their resting buds were small. The plants exposed to a 10-hour day, on the other hand, had enlarged the mother tubers to several times their original size, and they bore a large number of prominent resting buds but only a very few new tubers were formed. The new tubers, however, were noticeably larger than those formed under the full day length. The total weights of all tubers under the long-day and short-day conditions were 625 and 383 gm., respectively. These results are of special interest in bringing out the difference in mere enlargement of a storage organ, on the one hand, and the organization of new units, on the other hand. Apparently under the shortened illumination period there was a dearth of some formative material other than storage forms of carbohydrate. The original or mother tubers when grown a second season under a 10-hour day continued to enlarge as in the first season, attaining an average weight of 7½ ounces. It is of interest to note that the behavior under the 10-hour day closely approaches that of the related species *Apios priceana* Robinson, occurring in Kentucky. Under the natural day length of summer the latter species forms only a single tuber, which attains large size.

A bean from South America (*Phaseolus multiflorus* Willd). planted May 13, was exposed to regulated day lengths beginning June 3. Under the full day length of summer only moderate growth was made and there was no flowering. In October these plants were transferred to the greenhouse, electrically illuminated from sunset till midnight, and under these conditions growth was more vigorous than in summer but there was no flowering. Under a 12-hour day the plants behaved much like the preceding controls and there was no flowering. These plants were transferred to the greenhouse not provided with electric lights October 15. A few blossoms appeared November 29 and two seed pods matured. Under a 10-hour day flower buds were showing by July 15 and a number of blossoms opened, though no seed developed. Sparse flowering again occurred in September and in November. The plants were transferred to the greenhouse in October with the 12-hour day plants. The plants of the three series were taken up and photographed January 18. As shown in Plate 6, A and B, the roots of the plants exposed to 10 hours of light in summer and the short day length of winter were strongly tuberized, while under the long day length of summer and the artificially lengthened daylight period of winter there was no tuberization at all. The plants exposed to a 12-hour day in summer and the natural day length of winter showed only moderate tuberization of the roots. Without going into details it may be stated, also, that cuttings of two varieties of dahlia made May 10 were grown during the summer months in the greenhouse with and without electric illumination at night. Under the lengthened illumination period there was almost complete elimination of the usual formation of tubers.

Cinnamon vine, *Dioscorea divaricata* L., was grown under regulated day lengths, both from aerial tubers and from 1-year-old underground

tubers. The details of the experiments are summarized in part in Table VI. All plantings were harvested September 26. Cinnamon vine, like Peking soybeans, responds very promptly to the decrease in length of day beginning with the summer solstice, only that the response takes the form of tuberization of aerial axillary buds instead of flowering. Under the natural length of day the aerial tubers appear one month after the summer solstice, and this holds true for the progenies of both the aerial and the larger subterranean tubers. By exposure to an artificially shortened day length of 13 hours or less, beginning at the summer solstice, the appearance of aerial tubers was hastened by about two weeks. As is to be expected from the quick response to the first shortening of the day length following the summer solstice, the maximum effect in hastening the aerial tuberization is produced by a 13-hour day, further reduction in day length to 12, 11, or 10 hours being without effect. By exposing the plants to the reduced daily illumination period earlier in the season the date of appearance of aerial tubers is advanced accordingly. When the treatment is applied in the earlier stages of development, however, a longer period of time is required for the tubers to come into evidence than when considerable advance growth has been made. In all cases growth of the vine is quickly checked by exposure to the reduced day length. The effect is so pronounced that exposure to a length of day of 11 hours or less causes dying back of the young tips of the vines. This dying back practically ceases with a day length of 12 hours. It was not practical to obtain actual weights of aerial tubers produced under the different conditions of illumination, but it was observed that these tubers were formed in greatest number and of largest size under the 10-hour and 11-hour daily light exposure. Under a light period of 12 hours or more there was increasing tendency toward continued vegetative growth and reduced production of aerial tubers. There seem to be no marked, consistent differences in ratio between the weights of underground tubers and vines under the various light exposures. The underground tubers produced larger vines than the aerial tubers under the shorter light periods.

TABLE VI.—*Effect of differences in length of day on tuberization in cinnamon vine*

Kind of tubers planted.	Number of plants grown.	Date of germination.	Date of beginning light treatment.	Length of day to which exposed.	Date of first appearance of aerial tubers.	Average weight of tubers and vines.	Average weight of green vines.	Average weight of tubers.	Ratio of tubers to vines.
						Ounces.	*Ounces.*	*Ounces.*	
Aerial	1	Mar. 22	May 2	8 hours	June 20	4	1½	3½	7:1
Do	3	...do	...do	10 hours	...do	10	1	9	9:1
Do	1	...do	June 21	...do	July 4	6	2	4	2:1
Do	2	...do	July 23	11 hours	July 3	11½	3	8½	2.8:1
Do	1	...do	June 21	...do	July 2	6½	1½	5	3.3:1
Do	1	...do	...do	12 hours	July 7	3½	1	2½	2.5:1
Do	1	...do	...do	13 hours	July 2	4½	1	3½	3.5:1
Do	5	...do	Controls.	Full day length.	July 21	16	4	12	3:1
1-year-old underground.	1	Apr. 27	Mar. 22	8 hours	June 15	5½	1½	4	2.7:1
Do	1	Apr. 26	July 23	...do	July 22	19	9	10	1.1:1
Do	1	Apr. 27	Mar. 22	10 hours	June 23	8	2	6	3:1
Do	1	Apr. 21	June 21	...do	July 2	21½	8½	13	1.5:1
Do	1	Apr. 26	...do	11 hours	July 8	17	7	10	1.4:1
Do	1	May 4	...do	12 hours	...do	19½	3½	16	4.6:1
Do	1	Apr. 25	...do	13 hours	June 30	18½	4½	14	3.1:1
Do	3	Apr. 26 to May 29.	Controls.	Full day length.	July 21 to 23.	18½	7	11½	1.7:1

Tests were made with two varieties of yam (*Dioscorea alata* L.) furnished by the office of Foreign Seed and Plant Introduction, Bureau of Plant Industry. In each case two plants were grown in a box 3 feet long, 10 inches wide, and 10 inches deep. For plantings of the variety S. P. I. 46801 small root tubers were used, and for the variety S. P. I. 47001 aerial tubers were employed. All plantings were made May 14 and all tubers germinated between May 23 and June 4. The different light exposures began at time of planting. The yams were harvested October 6. Under a day length of 10 hours the yam S. P. I. 46801 developed at first a very marked chlorosis, the leaves taking on an almost pure yellow color, but by June 25 there had been a decided change toward the normal green. None of the plantings of either yam flowered in these tests. The variety S. P. I. 46801 formed no aerial tubers under the 10-hour exposure, but with 12 hours of light daily and under the full day length these tubers appeared. The variety S. P. I. 47001 formed no aerial tubers in any case. The relative development of vines and underground tubers under the different conditions are shown in Table VII.

TABLE VII.—*Behavior of two varieties of tropical yam under daily illumination periods of different duration*

Duration of illumination period.	Combined weights of vines and tubers.	Weight of tubers.	Weight of vines.	Percentage of tubers.
S. P. I. No. 47001:	*Ounces.*	*Ounces.*	*Ounces.*	
12 hours	126	81½	44½	64.7
Control; full day length	81	11½	69½	14.1
Do	79	11	68	13.9
Do	67	9	58	13.4
S. P. I. No. 46801:				
10 hours	117	86½	30½	73.9
12 hours	117	88	29	75.2
Control; full day length	109	41	68	37.6
Do	91	39	52	42.8
Do	106	55½	50½	52.3

It is quite clear that the shortened length of day caused a pronounced increase in tuber formation. The relative sizes of tubers formed by variety S. P. I. 47001 under a 12-hour day and the full summer day length are shown in Plate 10, A. It should be mentioned that while the plants exposed to the shorter day lengths formed only single thickened roots the controls tended to form more than one thickened root each.

Tubers of artichoke, *Helianthus tuberosus* L., were planted April 25 and were up May 5. Under a 10-hour day flower buds could be seen by June 21. The first evidence of flowering was a branching of the stem followed by formation of the flower buds. These buds, however, never developed to the flowering stage and by July 28 had been absorbed. By August 15 the plants were dead, having reached a height of about 2 feet. On the two control plants flower buds were showing August 30, and on one plant the first open blossoms were seen on September 22, while on the second plant the blossoms had not opened on October 1, when all plantings were harvested. Under the 10-hour day the plants

were small and appeared weak at all stages, but the two individuals together formed 35 tubers weighing 2 pounds and 4 ounces. Of the two controls, one reached 6 feet in height and the second 3 feet. The total number of tubers was 60, but these weighed only 1 pound and 1 ounce. Under the short-day exposure tuberization might be said to have been complete; that is, there were no elongated underground stems. As already stated, flowering could not be successfully accomplished so that there was no diversion of formative material toward seed formation. Under the natural range in length of day, on the other hand, there were many long underground stems with nodes only slightly tuberized, and in some instances no thickening of nodes at all could be seen. The tubers formed under the two conditions were strikingly different in appearance, as can be seen in Plate 7, A and B.

In the second series of tests with blazing star previously described (p. 876), it was observed that under the shortened daily light exposures well-developed corms of considerable size were formed, while the control plants, though many times larger in size, formed small corms or none at all.

Under the influence of a day length shortened to the point where successful flowering and fruiting is almost completely inhibited even the nodes of both primary and secondary stems of soybeans may be partially tuberized, as is shown in Plate 9, B. The arrested development of the flower buds accompanying the thickening of the stem is plainly seen.

The behavior of radish and beet under different day lengths has already been rather fully discussed (p. 887), and it only remains to point out here that under a short daily illumination period these plants continue to store up carbohydrate for a more or less indefinite period, eventually forming enormously thickened roots but no primary stem.

SIGNIFICANCE OF TUBERIZATION AS A FEATURE OF PHOTOPERIODISM

The data which have been presented will suffice to show that the prevailing length of day is an important factor in the formation of bulbs and tubers, as well as in flowering and fruiting. These data clearly indicate that the length of day not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material. Under the optimal illumination period for apogeotropic development there is normally no accumulation of unused carbohydrate, this material being utilized as rapidly as formed in the development of structures or tissues incident to maximum increase in stature. Under these particular conditions it may well be that the quantity of carbohydrate produced is a chief limiting factor in rate and extent of stem elongation. Any departure from this optimal light period will check stem elongation; and the first outstanding result is initiation of flowering; that is, the growing point is transformed into an inflorescence. The natural result of checking stem elongation is a tendency toward accumulation of carbohydrate. In successful fruiting this carbohydrate (in many cases first converted into oil) is stored in the seed and its coverings. Thus, in a special sense, the seed may be regarded as a tuberized structure. From the standpoint of nutrition, apparently one of the functions of fertilization as a prerequisite for successful setting of the fruit is to aid in setting up a "center of attraction" about which nutrient materials will be mobilized. Since there may be an optimal

light period for sexual reproduction which tends to direct the energies of the plant quantitatively toward flowering and fruiting (see p. 881), it seems possible that in some species exposure to this optimal light period will be found to favor successful fruiting without the aid of fertilization. In other words, the duration of the light period may be a factor in parthenogenesis and in the development of seedless fruits.

As the length of day is removed still further from the optimum for stem elongation, flowering and fruiting tend to give way to formation of the usual forms of vegetative reproductive structures in which tuberization is a characteristic feature. Tuberization marks a further step toward complete elimination of stem elongation. There is no hard and fast line between flowering and fruiting, on the one hand, and formation of tuberized vegetative reproductive structures on the other; and an intermediate day length may permit of both types of development just as vegetative stem elongation and sexual reproduction may proceed simultaneously. Various steps in the complete separation between sexual reproduction and the formation of tubers, however, are readily traced in the experimental data which have been given. In the soybean a sufficiently reduced light period which marks the end of successful flowering and fruiting likewise marks the beginning of tuberization in the stem. With Irish potato a daily light period of 18 hours caused vegetative development almost exclusively, for, although flower buds appeared, they were unable to unfold, and tuber formation was completely inhibited. Under the natural seasonal day length of 14 to 15 hours the plants flowered freely and there was moderate tuber formation. With 13 hours of light there was a decided increase in quantity of tubers formed, while with 10 hours of light there was very intense tuber formation, the ratio of tubers to "tops" being increased sevenfold as compared with the plants exposed to a light period of 13 hours. With a light period of only 5 hours there was a sharp decline in the ratio of tubers to tops, indicating, perhaps, that the income of carbohydrate by photosynthesis was but little in excess of immediate requirements for the limited growth which the plant could make. The results obtained with *Apios tuberosa* and with *Helianthus tuberosus* show, also, that the type of tuber formed may be markedly affected by the duration of the light period.

Associated with the change from flowering and fruiting to tuber formation there is a tendency toward the transfer of growth activity from aerial to underground parts of the plant. Thus, underground tuber-forming stems tend to replace aerial flowering stems. As the light period is further shortened even the elongation of underground stems is checked, with the result that the number of tuberized reproductive structures is reduced while their size is correspondingly increased. The final stage is reached in those cases in which tuberization is confined to the mother stem, as in the beet and radish when exposed to the action of a light period of short duration. In this case stem shortening has been pushed to the limit and the plant consists essentially of a rosette of leaves connected with the root system by means of an enormously thickened stem entirely lacking in power to elongate or to form aerial or subterranean branches.

The available data indicate that there is an optimal light period for tuber formation just as there are optimal periods for stem elongation and for sexual reproduction. The highest proportionate production

of tubers occurs with a light period which admits of only very limited stem growth. The absolute maximum production of tubers, however, is obtained with an intermediate light period, or, as actually occurs each year in higher latitudes, with a light period favorable to extensive stem growth followed by a period favorable to intensive tuber formation. In all cases thus far studied typical tuber formation takes place as a result of decrease of the daily light period below the optimum for stem elongation so that this type of tuberization is greatly favored by the shortening of the day in fall following the long summer days of high latitudes. Bulb formation, as illustrated by the onion, on the other hand, takes place as the result of increase of the daily light period above the optimum for stem growth so that formation of these structures is favored by the long days of summer which occur in high latitudes.

The results of the experiments in tuberization seem to show that the remarkable regulatory action of change in the length of day on stem elongation and various associated phenomena cannot be due directly to change in the quantity of carbohydrate formed through photosynthesis. The content of plastic carbohydrate in the plant at any given time, of course, will depend on the relation between rate of formation through photosynthesis and the rate of consumption in promoting growth and other vital functions. Tuberization furnishes visible evidence that under the prevailing conditions production of total carbohydrate is in excess of current consumption except in so far as local tuberization may be the result of mere transfer of preformed storage material from other parts of the plant. There seems to be little doubt but, beginning with the optimal day length for stem growth, decrease in length of day in general involves decreased formation of carbohydrate. It appears, however, that progressive loss of power to grow as a result of decreasing day length advances more rapidly than would be required from the decreased formation of carbohydrate as a controlling factor. It is necessary, therefore, to conclude that the duration of the light period exercises a regulatory action on internal processes of the plant other than those which merely determine the total quantity of carbohydrate produced. It remains to be determined whether the additional action is to regulate the form of carbohydrate produced or to control other processes not so clearly connected with carbohydrate formation. In any event there is convincing evidence that the duration of the daily light period is an important, and often a controlling, factor in determining whether the plant may use the product of photosynthesis as rapidly as elaborated in promoting growth or, instead, must store the material, perhaps for its own subsequent use or for the nutrition of its sexually or vegetatively produced progeny.

CHARACTER AND EXTENT OF BRANCHING

One of the characteristic effects of altering the light period from optimum to suboptimum for apogeotropic response is to promote branching. This increased tendency toward branching is frequently associated with flowering, a fact which is not surprising since both are promoted by a change in the same direction in the duration of the light period. Branching and flowering are not always associated, however, for not merely the direction but also the extent of the change in the light period must be taken into account. Apparently the most favorable light period for aerial branching lies between the apogeotropic optimum and that

period which is so far removed from the optimum as to permit only the leaf-rosette type of development, with complete inhibition of vertical elongation of the stem. Moreover, both the character and the extent of the branching appear to be influenced by the seasonal range in length of day.

Under the influence of long days cosmos continues to elongate the major axis, more commonly with no branching and even with a minimum of leaf development. Shortening the day length sufficiently to induce flowering also invariably causes branching at the top of the plant, as in the experiment discussed on page 883. In Table II data are given on the extent of stooling noted in the small grains. It is clear that in the spring types of wheat, rye, and barley stooling was greatly increased under the relatively short, natural day length of winter as compared with the artificially lengthened light period. This condition is reversed in the winter wheat and rye. This difference in response doubtless depends on fundamental differences in light requirements between the winter and the spring types of the small grains. The Honey variety of sorghum was sowed May 21 in the greenhouse. Under the natural day length of summer the plants began shedding pollen August 19, and as the days shortened there was marked stooling, many of the new basal shoots attaining the height of the original stem. A similar lot of plants receiving electric light from sunset till midnight began shedding pollen August 6. In this case the branching was confined chiefly to the tops of the plants, a behavior resembling that of cosmos. These differences in habit of branching are shown in Plate 11, A and B.

Rosettes of *Oenothera biennis* L., transplanted and placed under a 10-hour day beginning March 29, showed marked basal branching while the controls exposed to full daylight developed only a primary axis. This difference in behavior is shown in Plate 12, A. In the earlier stages the basal branches showed a prostrate type of growth but eventually showed a decided apogeotropic form of growth, as may be seen in the illustration. Returning to the final stages of development of the radish plant which had been long maintained under short-day conditions, as described on page 888, the influence of the short-light period on the growth of the branches is striking. Ordinarily the flowering stem of the radish branches chiefly at the top, as did the present plant at the outset. (Pl. 3, A.) As a result of the shortened day length to which it had been recently transferred the basal branches gained the ascendency and the plant gradually assumed the general shape and the type of branching of a shrub. The light-darkness ratio was such that the accumulated nutrient material could be used only for forms of growth involving a minimum of apogeotropic response. Finally, the pendent form of elongation of the primary and secondary stems assumed unusual prominence, producing a sort of "weeping-willow" effect, and marking the last phase of development. Apparently, there was at the end almost complete loss of the power for apogeotropic development. (Pl. 3, B.)

The duration of the daily light period also is a factor in the elongation of underground stems. As stated on page 876, Rumex is unable to develop flowering stems when exposed to a short day length. Aerial development is limited to the formation of leaf rosettes, as shown in Plate 12, B. In this case, however, there is very active underground development of stems, indicating, perhaps, a further step toward complete loss of the power of growth against the force of gravity. It may be surmised,

therefore, that the length of day is a factor in the so-called "law of level" applied to underground stems. Thus, in effect, a change in day length from optimum to suboptimum for apogeotropic stem elongation may cause the apical bud to lose its dominance and lateral buds to become active. As stages in the response to departure from the optimum light exposure for increase in stature may be noted branching at the top, middle, or base of the aerial portion of the axis; various degrees of erectness in the resulting branches, from the upright to the pendent form; the prostrate or creeping form of stem elongation; and development only of the underground type of stem which, in turn, may change its direction of growth upward or downward with a change in the daylight period toward or away from the optimum for negative geotropism.

ROOT GROWTH

Preliminary observations indicate that the duration of the daily illumination period may exert a marked effect on the relative development of the root and the aerial portions of the plant. For example, a cutting of Biloxi soybeans made no top growth at all through the winter months and the original leaves assumed a very dark color and generally unthrifty appearance. Apparently new buds were unable to develop. Upon examination of the underground portion of the plant in the spring it was seen that the soil contained a large mass of roots altogether out of proportion to the top of the plant, as judged by the usual summer growth. Other similar cases have been observed in which a light duration unfavorable to aerial development has caused extensive root growth. Growth of root and shoot, therefore, are not necessarily contemporaneous with respect to season, and arrested development of the exposed portion of the plant caused by suboptimal light duration need not be accompanied by checking of root growth.

PUBESCENCE

A feature of the experiments with *Amaranthus hybridus* L. described on page 873 worthy of note is the fact that under the shortened light exposures of 5, 10, 12, and 13 hours daily the leaves were glabrous and the veins were not prominent. The leaves of the control plants, exposed to the full-day length, on the other hand, were noticeably pubescent and the veins were decidedly more prominent.

Specimens of wild lettuce (*Lactuca spicata* (Lam.) Hitchc.) were transplanted to boxes from the field March 29 when in the form of small rosettes. Under a 10-hour day the first blossoms opened August 24, and under the full summer length of day the first blossoms opened August 21. The average heights were 65 and 96 inches, respectively. Under the shortened light exposure the reduction in stem elongation was accompanied by a marked increase in the size and in coarseness in texture of the leaves, as shown in Plate 10, B. There was, also, a striking increase in the pubescence and roughness of the leaves and stem under the short daylight exposure.

Thus, in the case of the short-day plant Amaranthus pubescence was not favored by shortening the light period, but in the case of the long-day plant Lactuca the shortened daylight period was markedly effective in this respect.

PHYSIOLOGICAL TRANSLOCATION

As was brought out in the preceding discussion of apogeotropism and of branching, a day length sufficiently below the optimum for apogeotropic expression often results in the leaf-rosette type of development (or prostrate form of stem growth), with more or less complete inhibition of upright stem elongation. Again, where upright growth of the primary axis has already taken place a change to suboptimum in the light duration tends to break down the dominance of the apical bud over the lateral buds, thus causing development of either aerial or subterranean secondary stems or branches, depending on the species in question and on the extent of the change in light duration. A third important phase of apogeotropism now requires consideration. Under the influence of the apogeotropic stimulus the lower parts of the plant, including leaf, stem, tuber, and thickened root, may yield up their nutritive constituents to the upper plant parts and for the most part tend to pass into a state of senescence, often followed by death. Thus, under the influence of a favorable light exposure the plant in increasing its stature may use over and over again, within limits, elements of plant food which may be available only in limited quantities. Conversely, under an unfavorable light duration the movement of plastic nutrient material is downward rather than upward, with resultant increased growth of the lower plant parts (branching, stooling, stolon formation, development of rootstocks, growth of roots, etc.) or, with a still less favorable light duration, formation of underground storage and resting organs. With only a moderate departure from the optimal light duration for increase in stature the effect may be to initiate flowering. In this case translocation toward the apex is not interrupted unless the change in the illumination period is too great. In the latter event the movement of food materials is diverted downward and successful fruiting is prevented, as, for example, in poinsettia exposed to a 10-hour day (see p. 876).

In a recent investigation Schertz (*21*) concluded that mottling of the older leaves of *Coleus blumei* is due to a failing nitrogen supply. There can be no doubt of the fact that with only a limited supply of nitrogen available there is often a decided tendency for the lower leaves to yellow and wither, while the upper portion of the plant continues to grow. For example, it is well known by tobacco growers that too low a proportion of nitrogen to phosphorous in the soil will cause "firing" or dying of the lower leaves of the tobacco plant. It still remains, however, to explain why the lower, more mature leaves yield their nutrient materials to the upper portion of the plant when the available supply of these nutrients is limited. It seems reasonable to suppose that this phenomenon is largely a manifestation of apogeotropism, and it appears that appropriate duration of the daily illumination period is a controlling external cause of this manifestation, whatever may be the internal mechanism through which it operates.

Thus, when exposed to a long day Cosmos has attained a stature and size out of proportion to the small volume of soil supporting the root system in the container. Under these conditions, however, the lower leaves soon perish so that only a comparatively narrow zone of living foliage immediately beneath the growing part follows the upward growth of the stem. In similar manner the lower portions of the stem tend to become woody and retain only minimal quantities of the plant food elements needed by the upper growing portion. The conclusion that

under the long-day influence there is active upward movement of nutrients from the lower portion of the plant seems to be supported by the fact that cutting back the stem quickly results in a swelling of the upper residual node, followed by rapid growth of branches (Pl. 17, A), an effect which is not observable under short-day conditions. Poinsettia shows even more markedly than Cosmos this tendency to continue upward elongation of the stem under the long-day stimulus in spite of a limited supply of nutrients from the soil, and, in doing so, to constantly shed the lower leaves as stem growth proceeds (Pl. 19, B).

For successful fruiting, after flowering has been initiated, it is essential, of course, that the reproductive structure receive from other plant parts an adequate supply of food materials. It is apparent that ordinarily such a supply of nutrients is assured under a day length approximating the optimum for initiating flowering. Under a length of day veering toward the optimum for apogeotropic response the direction of the food stream is not changed but the tendency is for diversion of the arriving food material to the development of vegetative structures, thus causing shedding of the blossoms or young fruit. A change in length of day in the opposite direction, however, if sufficiently marked will tend to direct the food stream downward, thus favoring the development of lower branches, leaves, bulbs, tubers, and roots. In some cases there appears to be an intermediate condition in which the food stream still moves to the upper parts (or is arrested before passing downward), but the light relations will permit neither vegetative nor floral buds to grow. Under these conditions aerial tubers may result, or in perennials the food material is simply deposited in the stem in the region of resting buds.

PIGMENT FORMATION

It is well known that continuous exposure to darkness for considerable periods of time ordinarily leads to etiolation. According to Bonnier (*3*) continuous exposure to electric illumination of an intensity much lower than that of sunlight causes a much greater development of chloroplasts and formation of chlorophyll than under normal conditions. In view of these facts the effects of differences in the length of the daily illumination period are striking. Exposure to a very short day length commonly leads to well-defined etiolation, but in some cases there is, after a time, more or less complete recovery, the tendency being toward a return to the normal green color. Under a 5-hour day turnip (*Brassica rapa* L.) became extremely etiolated and died after attaining a height of only a few inches. On the other hand, peanut (*Arachis hypogoea* L.) under similar conditions showed only slight evidence of etiolation at first and, soon becoming noticeably greener, made comparatively good growth throughout the summer. Sweet potato (*Ipomoea batatas* L.) became severely etiolated but later partially recovered and made fair growth. Soybeans behaved much the same as sweet potato. Irish potato showed severe etiolation and made relatively poor growth. *Aster linariifolius* L. retained its green color throughout. Under a 10-hour day *Dioscorea batatas* L. became markedly etiolated but subsequently almost completely recovered its normal green color.

In striking contrast with these etiolation effects, exposure to a length of day in excess of that producing etiolation but still below the optimum for flowering usually causes the leaves of the plant to assume a shade of green much darker than the normal. This effect has been noted in a

large number of species. While in a number of species there is a decided tendency toward eventual recovery from the condition of etiolation caused by a light exposure of very short duration, the reversed condition of increased depth of green color induced by a somewhat longer light exposure appears to be permanent so long as the proper light conditions prevail. This dark green color is associated with a condition of relative inactivity in the green portion of the plant so far as appearance goes, for typically there is little or no growth above ground. There is usually intense activity underground in this case in the formation and development of underground stems, tubers, and roots.

It is hardly necessary to discuss at length the fact that the changes in color of foliage leaves which are regularly associated with autumn leaf fall may follow in a natural manner as a sequel to flowering, fruiting, and other phenomena leading up to senescence, dormancy, or death of the plant when these processes are developed through artificial control of the light period. It will suffice, perhaps, to give a single, very interesting case illustrating the fact that color changes affected by the light duration are not confined to the chlorophyll pigments. A specimen of poinsettia forced into flowering by exposure to a 10-hour day, which was accompanied by the usual rich coloration of the bracts of the inflorescence, as described on page 876, was transferred September 2 to the greenhouse electrically illuminated from sunset till midnight. Under the influence of the lengthened illumination period a tendency toward replacement of the red color of the bracts by chlorophyll green soon became evident. The bracts persisted for many weeks, the younger ones assuming a clear green color. Finally, in December vegetative growth was resumed at the extreme tip of the inflorescence. The first growth of the new shoot was of the weak, pendent type, and the stem was red in color instead of the normal green. Eventually, however, the stem assumed upright growth and developed the usual green color. These color relations are well shown in Plate 1.

ABSCISSION AND LEAF FALL

In the case of such annuals as regularly shed their leaves before dying abundant evidence already has been given of abscission and leaf fall as phenomena following flowering and fruiting induced by suitable regulation of the length of day. Soybeans furnish a good example. Under the proper length of day flowering and fruiting are promptly followed by yellowing of the leaves, which take on a rich golden yellow color. Abscission is soon completed and, as a result, the stalks are practically bare of leaves when final maturity is reached. These effects are clearly shown in the previous article (7, *Pl. 66 and 69*).

Specimens of smooth sumac (*Rhus glabra* L.) were transplanted to wooden boxes in April and allowed to grow under outdoor conditions during the summer. On September 8 one lot was transferred to the greenhouse electrically illuminated from sunset till midnight while the second lot was placed in the greenhouse receiving natural light only. Under the latter conditions the leaves soon colored, although leaf fall was slightly delayed and the colors were not as bright as normal for the species. Under the lengthened illumination period there was no autumn coloration or falling of leaves. With the added electric illumination of very low intensity no additional top growth took place, but the leaves assumed an abnormally dark green color which was retained

through the winter. Although resting buds had been organized at the bases of the leaf petioles, these did not develop, and the general appearance above ground was one of comparative inactivity. Eventually, however, new shoots emerged from the soil as offsets. It is apparent that the light conditions were such as to permit of limited vegetative activity, thus preventing leaf fall, but these conditions were unfavorable for strong apogeotropic response. The appearance of the plants in January under natural winter illumination only and with the added electric illumination is shown in Plate 13, A and B.

Similar tests were made with dwarf sumac (*Rhus copallina* L.). Under natural illumination only, the leaves were only slightly delayed in coloring and falling, the colors being somewhat less intense than usual, and the plants did not awaken from dormancy during the winter. With the addition of the weak electric illumination, coloring and falling of the leaves was delayed for several weeks, although eventually the leaves were shed and the plants became dormant.

Experiments were carried out with the tulip tree (*Liriodendron tulipfera* (L.) in a similar manner. In this case transfer to the electrically illuminated greenhouse in September was promptly followed by renewal of active growth of aerial parts with abundant development of new leaves. There was no definite period of leaf fall, only individual older leaves gradually dying back. There was no abscission, and even after the leaves were dead the petioles remained firmly attached to the stem. In this manner the plants continued active vegetative development till the experiment was discontinued at the end of 18 months without having shown any indication of abscission or definite period of shedding of the leaves. The control plants soon lost their leaves through abscisson and remained dormant through the winter. The appearance of the plants under the two conditions of illumination is shown in Plate 14, A and B.

Thus, in the three species considered it is seen that weak electric light (3 to 5 foot-candles) as a supplement to the sunlight of short winter days has ranged in effectiveness from simply delaying leaf fall to causing more or less complete resumption of normal vegetative activities.

In the foregoing experiments it is shown that natural shortening of the day length in fall and winter results in leaf fall through abscission. It is not to be inferred from these results, however, that abrupt change from a long to a very short day will accomplish the same result. It already has been pointed out (p. 902) that in a large class of plants exposure to an illumination period slightly longer than that causing etiolation leads to development of an abnormally dark green color in the leaves. Under these conditions flowering occurs with difficulty or not at all and visible evidence of activity is found chiefly at or below the surface of the soil. A somewhat longer illumination period, on the other hand, favors reproductive activities followed by leaf fall and dormancy or death. In such plants, therefore, abrupt change from a long to a very short day will not lead to shedding of leaves but will result in diverting developmental activities downward in the plant, the leaves remaining intact and doubtless functioning in some measure. The above-mentioned two sumacs and tulip and other woody species were exposed to a 10-hour day during the summer months. Under these conditions none of the plants shed their leaves but, instead, assumed a generally unthrifty appearance and ceased upward growth. The leaves became dark green in color and to the touch appeared to contain but little sap. These results indicate that a gradual

transition from the long days of summer to the short days of midwinter is required to induce abscission in these plants.

DORMANCY AND DEATH

In general, exposure of annuals to the optimal illumination period for flowering tends to induce rapid senescence and death. In the same way exposure to certain definite day lengths causes perennials to enter into a state of dormancy. Deciduous trees and shrubs, in which the laying down of resting buds on the stem precedes leaf fall, enter into a form of dormancy involving temporary weakening, but not complete loss, of capacity for apogeotropic functioning. Herbaceous perennials enter into a form of dormancy in which there is more complete loss of the apogeotropic function. In both cases there is loss of leaves and photosynthetic activity is mostly suspended. That the first-named type of dormancy may be prevented by maintenance of a relatively long illumination period is shown by the experiments with the tulip poplar described in the preceding discussion of abscission and leaf fall. That the second type of dormancy also may be prevented by maintaining a long illumination period has been shown in experiments with *Aster linariifolius*.

Too great a departure from the optimal illumination period for apogeotropic growth, however, may cause the plant to assume a sort of pseudo-dormancy in which the leaves acquire an abnormally dark green color and the general appearance above the ground is one of comparative inactivity. Annuals, as well as perennials, show this response. These considerations lead to the conclusion that there may be an optimal light duration for death as well as for dormancy, while on either side of this optimum there may be very considerable and long-continued activity by the plant. On the one side the response is primarily and characteristically apogeotropic. On the other side the general tendency is toward geotropic response, visible expression being confined mostly to the surface or underground portions of the plant.

The results of tests with five varieties of soybeans will illustrate the fact that exposure to a definite light period results in rapid senescence and death in annuals. The varieties known as Mandarin, Peking, Tokyo, Biloxi, and Otootan were planted May 31 to June 1 in boxes and germinated June 4 to 6. They were exposed to day lengths of 10, 11, 12, 13 hours, and to the natural length of day of summer. For comparison, a lot of each variety also was given 10 hours of light daily of low intensity by means of a shade of cheesecloth. The principal results of the tests are presented in Table VI. On September 8 the seed pods of all varieties were fully matured and dry under all reduced day lengths, but only those of the Mandarin variety had matured under the natural length of day. Under the shorter light period all varieties flower at practically the same time; but as the light duration is increased the dates of flowering draw apart, and under the full summer day length the differences in time of flowering become very great. As to the advance of senescence following the flowering and fruiting stages two facts are brought out in the table. It is evident that the rate at which senescence advances in a given variety depends on the duration of the light period and, also, it is evident that the optimal light period for advance of senescence differs with the variety. For the earlier varieties, Mandarin and Peking, it appears that the optimal period is approximately 13 hours, while for the very late varieties, Biloxi and Otootan,

the optimal is 11 to 12 hours. The Tokyo seems to be better able to resist the advance of senescence than the other varieties. The Peking is particularly responsive to a definite light exposure in this respect and under the 13-hour day began shedding its leaves early in August.

TABLE VIII.—*Behavior of different varieties of soybeans planted May 31 to June 1 and grown under daily light exposures of different lengths.*

Treatment.	Average height of plants on Aug. 5.					Time of first blossoming.				
	Mandarin.	Peking.	Tokyo.	Biloxi.	Ototan.	Mandarin.	Peking.	Tokyo.	Biloxi.	Ototan.
	In.	*In.*	*In.*	*In.*	*In.*					
10-hour day, 5.30 a. m. to 3.30 p. m.	18	13	15	18	23	June 27	June 25	June 27	June 30	July 1
11-hour day, 5.30 a. m. to 4.30 p. m.	20	14	20	20	25	...do....	...do....	...do....	...do....	Do.
12-hour day, 5.30 a. m. to 5.30 p. m.	20	16½	20	21	30	...do....	...do....	...do....	July 2	July 4
13-hour day, 5.30 a. m. to 6.30 p. m.	27	19	27	28	36	...do....	...do....	June 30	July 5	July 6
Controls, full summer day length	25	17	51	31	33	July 2	July 18	Aug. 8	Sept. 7	.....
10-hour day, 5.30 a. m. to 3.30 p. m., under heavy shade	18	11	15	18	33	June 28	June 27	June 28	June 30	July 2

Treatment.	Appearance of plants Sept. 8.				
	Mandarin.	Peking.	Tokyo.	Biloxi.	Ototan.
10-hour day, 5.30 a. m. to 3.30 p. m.	Leaves green	Leaves green	Leaves green	Leaves yellowing.	Leaves yellowing.
11-hour day, 5.30 a. m. to 4.30 p. m.	Leaves yellowing.	Most leaves green.	.....do.....	Mostly leafless.	Leafless.
12-hour day, 5.30 a. m. to 5.30 p. m.	Few green leaves.	...do.....	.....do.....	Few green leaves.	Few green leaves.
13-hour day, 5.30 a. m. to 6.30 p. m.	Leafless.....	Stalks dead, leafless.	.....do.....	Most leaves green.	Leaves green.
Controls, full summer day length.	Nearly leafless.	Leaves green	Green, still flowering.	Still growing.	Still growing.
10-hour day, 5.30 a. m. to 3.30 p. m., under heavy shade.	Mostly leafless.	...do.....	Leaves green	Few green leaves.	Leafless.

Considering that a light period which is unfavorable for vegetative activity may cause death or dormancy, as the case may be, the question arises as to the relation of the light period to emergence from dormancy. The line of division between death and dormancy is often a very narrow one, and there are varying degrees or conditions of dormancy. It appears that the time and conditions of emergence, or whether it shall occur at all, may depend in part upon the length of day under which the plant enters upon the state of dormancy. The behavior of the Kudzu vine (*Pueraria hirsutus* (Thunb.) Scheid.) in this particular is of interest. Beginning May 12, seedlings which had been growing since January 5 were given a 10-hour day through the summer while control plants were exposed to the full day length. Under the 10-hour day growth of the aerial portions of the plant soon ceased and the foliage assumed a very dark green color. The basal portion of the stem became considerably enlarged. The controls grew vigorously through the summer. Both lots of plants were transferred to the greenhouse September 15. In both cases the leaves gradually withered and fell and the plants became dormant, although this condition was not fully reached till early in December. By January 11 new shoots

began to appear on the plants which had been exposed to a 10-hour day during the preceding summer. The renewed growth was rapid and vigorous. The new foliage showed the abnormally dark green color of the original leaves which had developed under the 10-hour day. The control plants did not renew their growth till February 16 (Pl. 15, A and B). Beginning August 16, a third series of plants were placed in the greenhouse artificially lighted from sunset till midnight. In this case dormancy was inhibited and rapid growth continued during the winter months. Moreover, no woody stems were formed, even the tissues of the basal portions of the stem remaining soft, in contrast with the usual behavior of Kudzu.

As has been repeatedly pointed out, there is a well-defined quantitative aspect of the influence which the length of day exercises on the formation of seeds and vegetative resting and reproductive structures so that, other conditions equal, it is to be expected that emergence from the rest period will be materially influenced by the prevailing length of day under which the state of dormancy was developed. There can be no doubt that both the quantity and the character of the plastic "reserve" material available for subsequent needs of the resting embryo are conditioned in large measure by this factor.

As regards the direct action of length of day on emergence from the rest period, it would seem that successful emergence without the aid of a favorable day length will depend on whether suitable temperature conditions and other environmental conditions can set in action the necessary internal processes for renewing active growth. Since ultimately both the character and the extent of growth in the plant are dependent largely on the prevailing day length, the point at which this factor must intervene in the renewal of activity by the resting structure will be governed by the amount of potential growth energy that becomes available from stored materials under the existing environment. In the case of the vegetative bud the action of a favorable light period may come into play almost at the outset. What action, if any, the light period may exercise directly on nonchlorophyllous structures can not be stated at this time. It has been shown by MacDougall (*13*) that in certain cases resting buds attached to large storage organs may maintain renewed growth for a year or more without receiving any light. By way of contrast, however, the behavior of *Hibiscus moscheutos* L. is of interest. Under short day conditions this plant is unable to make appreciable growth (*7, p. 578*). Seedlings were grown in buckets during the summer under outdoor conditions and were left out of doors during the following winter. Beginning March 27, one series was allowed to receive only 10 hours of light daily while a second series continued to receive the full seasonal period of daylight. On these control plants new shoots apeared April 8 and 9 and growth continued in normal fashion. Under the 10-hour day a feeble development of new shoots was noticeable by May 18, but these soon died when still less than an inch long. The condition of the plants under the two conditions of light on June 21 are shown in Plate 17, B. Similarly, *Aster linariifolius* was transferred to the greenhouse October 17 and on November 27 was placed beneath a 100-watt electric light provided with reflector, at a distance of 1 foot, the light being on each day from 4.30 p. m. to 12.30 a. m. New shoots appeared during the third week in December. Under control conditions the rest period was not broken till spring. In the case of second-year beets, previously described (*p. 888*), although development of flowering stems began under a 10-hour day, the plants were never able to flower. A number of small trees and

shrubs, including *Acer negundo* L., *Acer rubrum* L., *Cornus florida* L., and *Rhus glabra* L., which were exposed to an 8-hour day, beginning early in January, successfully unfolded their leaves at about the same time as did the controls. Some of these, however, made but little subsequent growth even under a 10-hour day, which proved to be too short for their requirements. It appears, therefore, that there are all degrees of dependence upon the direct action of the light period in the matter of emerging from dormancy. Much, doubtless, depends on the conditions under which the plant enters dormancy and the environmental conditions which prevail during the rest period. The whole subject of dormancy in relation to the light period will require further study before general conclusions may be drawn.

SENESCENCE AND REJUVENESCENCE

All experimental data thus far secured seem to support the conception advanced long ago that a common cause of general senescence, that is, senescence of the whole or greater part of the plant, is the intense formation of independent or semi-independent reproductive structures. In annuals a length of day which favors intense flowering and fruiting has resulted in rapid senescence and death, and in herbaceous perennials this has been true with respect to both sexual reproduction and tuber formation. This relationship applies whether the optimal light period for reproduction corresponds to the longest summer days or the shorter days of spring and fall. It has been pointed out that Peking soybeans rapidly pass into senescence under the influence of a day length of about 13 hours, which approximates the optimal length of day for flowering and fruiting. Under a light period much in excess of, or much below, 13 hours senescence is deferred. *Convolvulus tricolor* L. furnishes an example of how a light period too short to admit of flowering may indefinitely delay decline and death. Seedlings exposed to a 10-hour day, beginning June 8, were unable to blossom, while growth continued for a period of more than eight months. In this instance the plant attains an abnormally large size by continued exposure to a light period too short to admit of flowering.

Localized senescence is a very common feature of photoperiodism and, of course, is frequently accompanied by regeneration phenomena. When Cosmos, for example, is exposed to a long day, continued elongation of the axis is accompanied by progressive loss of lower leaves. As the light period becomes less favorable for apogeotropic response the tendency is in the reverse direction. In various species there may be simple abscission and leaf fall, accompanied by formation of resting buds; branching, as a result of loss of dominance on the part of the apical bud, followed by decline of the latter; progressive decline of the upper portions of the primary axis; a general transfer of activity from above to the ground level or even to subterranean parts. Examples of all these stages have been presented in the preceding pages.

Since certain light periods may defer the appearance of senescence, the question arises as to whether exposure to these light periods may effect rejuvenation in cases of advanced decline and impending death. Examples of this type of rejuvenescence were given in the previous paper (7, *p.* 567) in the cases of soybeans, *Ambrosia artemisiifolia* L., and wild aster. *Bidens frondosa* L. also furnishes an excellent example.

As reported in the former paper, this species flowers precociously under the short days of winter but flowering is readily inhibited by lengthening the light period. The two phases of activity are represented in a single plant in Plate 16, A. Under the natural day length of winter this plant flowered early in February at a height of 5 inches. At the time of flowering the plant was transferred to the greenhouse electrically lighted from sunset till midnight. The original stem died to the ground, but under the lengthened light period new shoots appeared and these reached a height of more than 4 feet. It seems clear that in typical annuals not only may general senescence be deferred more or less indefinitely by preventing exposure to the particular light period which favors reproductive activities but even after this stage has been reached rejuvenescence may be effected through regenerative processes. The final stages in an interesting type of rejuvenescence in the inflorescence of poinsettia, the earlier stages of which are referred to on page 903, are illustrated in Plate 19, B.

LOCALIZATION OF THE PHOTOPERIODIC RESPONSE

An experiment was made with Cosmos to determine whether the stimulus imparted to a particular portion of the plant by change in the light period extends its influence to other parts of the individual. The results were clean cut, and some interesting facts were brought out. Young seedlings, propagated under long-day conditions, were cut back to the first node above the cotyledons so as to cause two opposite branches to develop. A month after the plants were cut back a cardboard screen was interposed vertically between the branches of each individual, the plane of the screen extending in north-and-south direction. On one side of the screen a 100-watt electric light, with reflector, was turned on each day from sunset till midnight. On the other side of the screen only the natural daylight period of the winter season prevailed. The plants were so adjusted with reference to the screen that the portion of the plant below the point of origin of the two branches was on the long-day side in some instances and on the short-day side in others. In all cases the branches exposed to the short day promptly flowered while those exposed to the long day continued the sterile, vegetative type of development, without regard to the exposure of the basal portions of the plant. Moreover, in all cases the sterile branch assumed a dominant influence over the basal portion; that is, the basal portion of the plant continued to enlarge in harmony with the growth of the vegetative branch while the flowering branch soon ceased growth and passed into a state of decline. These relations are clearly shown in Plate 16, B. In this connection it is to be recalled that the determinative influence of the light period on an aerial part may be transmitted to a subterranean part of the plant, as shown in the case of the potato. In this instance the apogeotropic influence of the long light period inhibited tuber formation and, instead, buds at the nodes of the stolons were caused to germinate without a rest period, thus forming offsets. The experiment with Cosmos shows that such influence on an aerial plant part may not be transmitted to another coordinate member, and to this extent perhaps the stimulus may be regarded as localized. It is not entirely clear as to how or why the branch exposed to the longer light period is dominant over the branch exposed to the shorter light period in its influence on other portions of the plant.

LIGHT PERIOD IN RELATION TO OTHER FACTORS OF THE ENVIRONMENT

To arrive at a satisfactory conclusion as to the practical significance of the seasonal range in length of day in plant growth it is essential, of course, to know to what extent other environmental factors are likely to modify the action of the length of day. Because of the unvarying regularity of the annual cycle in day length it is apparent that any influence of this factor on plant behavior will find expression in the normal or usual seasonal periodicity rather than in unusual or occasional responses. In short, the change in length of day may be expected to bring about the usual rather than the unusual in the annual cycle of the plant's activities. On the other hand, occasional severe drought, abnormally high or low temperatures, unusual chemical conditions of the soil, attacks by insects or diseases, may cause temporary disturbance either qualitatively or quantitatively in the usual seasonal development of the plant.

The question of chief interest in the present connection is the extent to which the normal sequence of seasonal changes in other climatic factors may modify or nullify the influence of length of day on periodicity in plant activity. That various climatic factors, as well as the factor spoken of as soil fertility, may produce quantitative differences is known to all. To what extent the actual annual range in these factors may cause distinct qualitative differences in expression is not so well understood. In connection with the present investigations a limited number of observations have been secured with certain species on the significance of climatic factors other than change in the light period in phenomena of plant periodicity.

As is well known, the seasonal change in the lengeh of day is accompanied by important changes in the intensity of the sunlight and in its quality, that is, in the energy distribution of the visible portion of the sun's spectrum. Hence, it is of special interest to consider these factors in relation to photoperiodism. In the earlier paper considerable data were presented (7, *p. 581 et seq.*) tending to show that reduction of the intensity of the sunlight in summer to as low as one-third the normal had no appreciable effect on the time of flowering of soy beans. These results have been confirmed in the experiments referred to on page 905. As a part of the tests a long, rectangular frame, with sides and top covered with cheesecloth, was erected over a portion of a steel track leading into the dark house so that a truck carrying one lot of the plants could be rolled under the shade each day while a second lot was exposed to the direct sunlight. A series of measurements of relative light intensity within and outside the shade from 10 a. m. to 3 p. m. on July 27 were made by R. A. Steinberg, of this office. For the purpose a Sharp-Millar model F photometer, tested by the United States Bureau of Standards and found to be accurate to between 5 and 10 per cent, was used. In round numbers the average intensity thus obtained for the period indicated was 10,500 foot-candles in the open and 3,500 foot-candles under the shade. The results of this test of the comparative effectiveness of light duration and light intensity are given in Table VIII. The maximum effect of a reduction in light intensity to one-third the normal, with a light period of 10 hours, was to delay the date of first flowering by one to two days. In marked contrast, a reduction in the length of the daily light period from the average natural summer day length of about 14½ hours to 13 hours, a decrease of about 10 per cent, promptly initiated

flowering in every case. The vegetative period of the Biloxi variety, for example, was reduced from 94 days to 21 days. In this experiment the reduction in the duration of the daily light period was no greater than actually occurs during the growing period of the Biloxi. The light intensity was reduced by shading to about 3,500 foot-candles, while the natural decrease up to the close of the growing season (October 1) only is to an average of about 6,200 foot-candles for the same hours of the day (9 a. m. to 3 p. m.), and to about 4,500 foot-candles for the entire 10-hour day (7 a. m. to 5 p. m.). It is to be noted, also, that the reduced intensity shows no selective action on the different varieties with respect to time of flowering, whereas this is the characteristic feature of the varietal response to change in light duration. The varietal responses to the different light periods, in fact, are strictly in accord with the natural behavior of the varieties as to earliness of maturity. There are significant differences, also, in the effects of change in intensity and change in duration of the light on the heights attained by soybeans. In the former paper (7) it was shown that under the natural day length of summer, reduction of light intensity causes increased elongation of the stem in soybeans and that, in fact, with heavy shading a typical viny form of growth develops. In Table VI it is shown, however, that under a 10-hour day heavy shading failed to cause increased stem elongation in comparison with plants exposed to the full sunlight for 10 hours daily, except in the case of the Otootan variety. The effect of shortening the duration of the light period is the reverse of that produced by reducing the intensity; that is, there is decided reduction in stem growth, as is shown in Table VI. Here, again, the effect of a shortened light period on the stature attained by the plant is in accord with the progressive decrease in stature which results when the date of planting is made later and later in the season, an effect well shown in the former paper (7, *Pl. 78, B*). Decrease in light intensity fails to account for either the initiation of flowering or the slowing down in stem growth in soybeans which occur with advance of the season after the summer solstice.

A similar experiment was made with the Silverskin onion, using the same shade that was employed in the test with soybeans. With the light intensity thus reduced to one-third the normal, plantings of the onion made June 16 grew and developed bulbs in substantially the same manner as in the open sunlight. At most, the dying back of the tops was delayed a week and the bulbs were about the same size as those of the controls. Under a 10-hour exposure to full sunlight, on the other hand, there was no bulbing and the tops remained green throughout the summer. These differences in behavior are well shown in Plate 18, A. There is no evidence, therefore, that bulb formation in the onion is due to excessive intensity of sunlight in the summer.

In the light of these experiments it would seem that use of such terms as "quantity of solar radiation" in relation to specific plant responses should be abandoned where such terms involve both intensity of the radiation and duration of the daily exposure. Not only is there frequently no approach toward equal effects for equal changes in these two factors but these effects may actually be in opposite directions. The futility of attempting to apply quantitative energy relations of this sort to the process of flowering in the soybean plant is further emphasized in the following data. Beginning June 30, one lot of Peking seedlings was placed in darkness from 10 a. m. to 4 p. m. daily; a second lot was simi-

larly darkened from 10 a. m. to 2 p. m.; while a third lot was excluded from the light only during the early morning period prior to 6.30 a. m. Controls were exposed to the full day length, averaging 14 hours and 33 minutes (sunrise to sunset) for the month of July. The dates of first flowering in the four series were August 5, August 5, July 27, August 10, respectively. It is estimated that in the first three series the reduction in sunlight by darkening amounted to approximately 65, 48, and 2½ per cent, respectively, of the total for the day. It is evident that a reduction of 2½ per cent in the amount of sunlight when applied in the morning was more effective in initiating flowering than a reduction of 65 per cent applied in the middle of the day. With respect to flowering, the remarkable sensitiveness of soybeans to change in length of the daily light period is emphasized in the behavior of successive field plantings of the Peking; those germinating, for example, on July 20 showed a vegetative period 8 to 10 days shorter than those germinating July 5, while the change in length of day in the interval of 15 days was only about 17 minutes, or 2 per cent. It should be noted in this connection that Rivera (*20*), as a result of his investigation, considers that the duration of the daily illumination period is more potent than the intensity of the light in influencing crop yields.

To obtain direct experimental evidence on the possible significance of the seasonal change in the quality or the composition of sunlight in the development of the plant offers considerable difficulty, although this phase of the subject is now under investigation. The indirect evidence available at this time seems to indicate that this factor is not of great importance, at least for the species under study. For example, flowering is easily induced in midsummer by shortening the day length through cutting off the morning and late afternoon sunlight. The plant in this case receives a maximum proportion of sunlight of the shorter wave lengths. Flowering also occurs, however, through natural shortening of the day length in fall and winter, and in this case the plant receives a greatly reduced proportion of the shorter wave lengths.

As is more fully brought out in the final chapter of this paper, it seems likely that the internal water supply of the plant is of fundamental importance in photoperiodism. Nevertheless there is no definite seasonal change in rainfall in the eastern United States which would account for seasonal periodicity in plant activities. It is necessary to distinguish between the internal mechanism of the plant concerned in regulating the water content, on the one hand, and the external water supply, on the other. In the previous paper (7) detailed data were given to show that wide differences in the water content of the soil were without appreciable effect in initiating flowering in soybeans. By reference to the experiments with *Sagittaria latifolia* recorded on page 878 it will be seen that growing this plant as an aquatic under a 10-hour day delayed flowering by only about two weeks as compared with cultures under mesophytic conditions. The reduction in the light period, on the other hand, shortened the vegetative period by more than two months.

Temperature undoubtedly is the most important environmental factor in relation to the action of the light period on plant growth, and two phases of its action need to be taken into account. In the first place, the general tendency toward an increased rate of growth and development as the temperature increases, within favorable limits, and the reverse tendency with decrease of temperature should be to delay or even inhibit flowering of typical summer annuals in late summer or fall.

For this reason decrease of temperature with advance of the season could not be expected to account for the initiation of flowering in soybeans in August and September, the tendency being in the opposite direction. Direct evidence on this point is furnished by plantings of the Peking and Biloxi varieties made in the greenhouse on December 14 under controlled conditions of temperature, with and without electric illumination from sunset till midnight. With a mean temperature of approximately 72° F. and a daily temperature seldom exceeding in either direction a range from 65° to 75°, the soybeans germinated December 19 and, with natural illumination only, the Peking began flowering January 25 and the Biloxi February 25. With the added artificial illumination, flowering was inhibited. With a mean temperature of approximately 55° and a daily temperature seldom exceeding in either direction a daily range from 50° to 60°, the soybeans which germinated December 27 had not flowered March 1 under either light exposure. In the first case the mean temperature (close to 72°) was somewhat below the mean temperature for the month of July at Washington (76°.8). In the second case the mean temperature (approximately 55°) was considerably below the Washington mean of 68° for the month of September. It is clear that the lower temperature delayed the time of flowering.

A second phase of the temperature effect seems to relate more directly to internal conditions of nutrition, especially affecting, perhaps, the balance between income and outgo of carbohydrate because of the change in the rate of respiratory activity. In this way the relation between the duration of the light period and the prevailing temperature may become of decisive importance. The requirements for successful flowering necessarily are more critical than for vegetative growth, since the floral structures are dependent on other parts of the plant for their supply of suitable forms of carbohydrate. Unfavorable conditions of balance, therefore, either because of reduced income or excessive outgo of carbohydrate, may easily inhibit flowering and fruiting. These conditions would not necessarily check development of the green parts of the plant, since such structures tend to be self-supporting with respect to carbohydrate supply. It need only be added that in these considerations the form of the carbohydrate as well as the quantity must be taken into account.

It has been demonstrated that for many species, probably the majority of them, the extremely short days of winter in higher latitudes will inhibit increase in stature, and in many cases flowering and fruiting also. Largely as a result of the transition from the longer summer days to the shorter days of winter many annuals are caused to die and many perennials are forced into a state of more or less complete dormancy. Hence, the income from photosynthesis is reduced to a minimum. It is obvious, therefore, that exposure to a relatively high temperature during the short days of winter may easily prove disastrous to the plant. The importance of exposure to cold during the winter rest period has been recently emphasized by Coville (6). Exposure to the forcing action of high temperatures in winter is likely to be especially unfavorable to reproductive structures. It is well known, also, that in some way exposure to cold may exercise a beneficial action on nonchlorophyllous resting organs, resulting eventually in renewed activity. In general, when the dormant structure has entered upon the rest period with the necessary "reserve" materials the initial processes of renewed activity may be stimulated by favorable temperature conditions, such as a period

of chilling. The extent to which these renewed activities may progress after they have been initiated in the absence of a favorable light period, however, is extremely variable, as was pointed out on page 907. There seems to be no question as to the importance of the relationship between the action of the seasonal decrease in length of day in forcing the plant into a state of relative inactivity, on the one hand, and the effect of the cold of winter in promoting successful emergence from the rest period by conserving and bringing into play the stored energy of the resting organ, on the other hand.

RANGE OF RESPONSE TO LENGTH OF DAY

It is apparent that species differ widely in their sensibility to change in the length of day and in the form of expression which the response may take. Cosmos continues elongation of the vegetative stem under any day length in excess of about 13 hours, while under all day lengths much below 13 hours flowering readily takes place. Mikania may maintain sterile vegetative growth under the extreme day length (in excess of 15 hours) of summer in high latitudes. It flowers readily under a daily light period of about 14 hours, while both growth and flowering are inhibited by a light period of 13 hours or less. Goldenrod forms a flowering stem under any length of day in excess of approximately 8 hours, but with a shorter light period only the leaf-rosette type of development is possible. Sorrel (Rumex) forms aerial flowering stems when exposed to the long days of summer, but under the short days of winter only the underground type of stem is formed. Artichoke is unable to flower under a light exposure of 10 hours or less, but under these conditions there may be intense tuber formation. Under a 10-hour day groundnut (Apios) is unable to flower, there is no elongation of aerial stems, the number of new underground tubers is greatly reduced, and the mother tuber is materially enlarged. Under a longer day length all of these forms of response in groundnut are reversed. The Mandarin soybean flowers under the longest summer days at Washington, while the Peking does not begin flowering till the latter half of July, the Tokyo about two weeks later, the Biloxi some three weeks later still, and, finally, the Otootan about September 15—all in response to the prevailing day length. In the violet the extreme seasonal range in day length from early spring to midsummer suffices only to change the type of blossom from the blue petaliferous to the cleistogamous form. Finally, in buckwheat flowering occurs throughout the range of day length from 5 to 18 hours, although under the shorter period it behaves as an ephemeral, and under the longer period it assumes the form of a giant everbloomer.

It may be considered that there is a wide range or "scale" of response to change in the light period and that the portion of this scale covered by the individual species or variety is extremely variable. For example, buckwheat through all ranges in length of day prevailing in the temperate zone occupies only that portion of the scale lying between the strictly flowering condition and the sterile, giant type of development. The bean referred to on page 893 occupies the portion of the scale represented in the strictly sterile giant form, the flowering condition, and the phenomenon of tuberization. No single species has been observed which manifests all forms of response, but one might perhaps conceive of a sort of composite plant, combining the responses of several different species and thus covering the entire range. Beginning with one of the extremes of this

range of response, there would be an optimal light period for elongation of the vegetative stem or increase in stature. Under this optimal light period, which in the first instance will be assumed to correspond with the longest days of summer, there would be more or less indefinite elongation of the primary axis, with no branching and a minimum of foliage development (Cosmos). Any departure from the optimal light period would check stem elongation; and, as a result, a chain of responses would come into expression as the light period became progressively less favorable for increase in stature. The first effect of outstanding importance would be flowering and fruiting; and, as a result of partial loss of dominance in the apical bud, branching might occur at this stage (Cosmos). The change in the light period may be such as to direct the plant's energies more or less quantitatively toward flowering and fruiting, in which case senescence and death would be the logical outcome, the plant behaving as an annual. Even in this case, however, rejuvenescence may be effected under favorable conditions by a return of the optimal light period for vegetative growth, the plant thus escaping death (Bidens). Between the respective optimal light periods for stem growth and for sexual reproduction there would be a buffer zone in which combined growth and flowering would occur, thus representing the everblooming condition (Viola).

With a further departure from the optimal light period there would be more or less complete inhibition of stem elongation, resulting in intense tuberization; that is, stem elongation would be replaced by stem thickening. In this case flowering would be inhibited (artichoke). If a primary axis had previously developed, there would be a general downward transfer of activity to the ground level or even beneath the soil surface. The end result would be the formation of underground resting structures, as rhizomes or tubers. Here, again, between the respective optimal light periods for flowering and for tuberization there would be an intermediate zone in which important responses find expression. There may be combined flowering and tuberization (groundnut). Aerial and underground types of tuberization are alternative forms of expression (cinnamon vine). Aerial branching may give way to underground branching (sorrel). The stemless leaf rosette, with or without tuberization, is an important type of activity falling within this zone. Here would be placed the flowering herbaceous perennial which flowers and fruits so freely as to cause the death of the aerial parts but with sufficient regenerative energy remaining to organize underground perennating structures. The final response would be death as a direct result of starvation when the light period has become too short to furnish sufficient photosynthetic material to support any form of activity. The circumstances of death in this case are quite different, however, from those which induce intensive fruiting and thereby result in the death of the mother plant. It is to be understood, of course, that all forms of expression are conditioned on the existence of general environmental conditions falling within the limits of tolerance of the species in question.

The above-mentioned series of responses would result from a progressive decrease in the duration of the light period. Assuming an optimal light period for stem elongation corresponding to the intermediate day length of spring and fall, a somewhat different chain of events, not yet fully determined, seems to result from exposure to light periods of excessive length. Probably one of the first notable effects of a light period in

excess of the optimal for stem growth is the laying down of flower buds. It is possible, also, that resting vegetative buds of woody perennials result from these conditions, although this has not been definitely determined. It has been shown that bulb formation commonly results as one of the final effects of an illumination period of excessive duration. No example of death from this cause has yet been encountered.

These outlines of the series of responses initiated by graded departures from the optimal light period for stem growth are necessarily tentative. All responses are not included, and it is possible that the proper sequence of those which have been given will ultimately require modification in some instances.

NATURE OF THE REGULATORY ACTION OF LENGTH OF DAY

Definite conclusion as to details of the internal processes concerned in the phenomena of photoperiodism is withheld, pending the completion of fairly extensive biochemical investigations now in progress in this office. For the present only the more general aspects of the problem as brought to light in the response of the plants as a whole will be considered. These various forms of response seem to indicate clearly that in some way the internal water supply, the degree of hydration, of the living cell content is subject to very delicate regulation by change in the ratio of the number of hours of light to the number of hours of darkness in the daily period of 24 hours. It will be observed that there is an optimal light-darkness ratio for maximum rate of stem growth, and Chandler (*5*) and Reed (*19*) have shown that maximum growth is correlated with a dilute cell sap or high degree of turgidity. In recent years MacDougall (*14*) has especially emphasized the significance of hydration in growth phenomena. The various responses of the plant coming into expression as the light period becomes progressively less favorable for upward stem growth are practically without exception those more or less associated with relatively xerophytic conditions. Tendency toward increased flowering and fruiting, increased branching, pubescence, abscission, and leaf fall, the general tendency toward stem shortening, tuberization, and increased underground stem development are phenomena in point.

The behavior of *Viola papilionacea* under different light periods furnishes direct evidence on this subject. During the shortest days of winter the plants are in a semidormant condition, the young leaf buds becoming tuberized through loss of power to elongate the leaf petiole. Under the intermediate day length of spring leaves with short petioles and blue blossoms with relatively short stems make their appearance. During the long days of summer the petioles of the leaves are greatly lengthened. When plants bearing the summer type of leaf are abruptly transferred to short-day conditions partial collapse occurs within two or three days, obviously due to loss of turgidity in the leaf stem. In due course, also, the characteristic spring type of blue blossoms reappears. This behavior is shown in Plate 18, B.

Without at this time raising the question as to the exact mechanism of the internal processes affected, it seems possible that highly refined regulation of the degree of hydration of the protoplasm furnishes a basis for the various responses of the plant to changes in the light period. It is believed, however, that this regulation is effected primarily through direct action on the internal mechanism which maintains hydration rather than by merely influencing external factors which modify relative

gain and loss of water. This implies, perhaps, control of permeability. Proper distinction must be made, therefore, between regulation of the external supply of water and control of the internal mechanism involved in dealing with phenomena of hydration. It seems probable that the light period, acting through control of the internal mechanism, is capable of exercising a far more refined regulation of the internal water supply of the plant's parts than is ordinarily accomplished by change in the external supply of water. The experiments with Sagittaria recorded on page 878 indicate that this is true. Growing this plant as an aquatic at most delays flowering by some 10 days as against growth under mesophytic conditions, while appropriate change in the light period resulted in sharp response. As already pointed out, growing soybeans under semixerophytic conditions in contrast with mesophytic conditions was entirely without effect in initiating flowering. It may occasionally happen, perhaps, that the available external supply of water will be just right to favor a definite response such as successful flowering. It seems likely also that plants which are not particularly sensitive to change in the light period, including typical everbloomers, such as the cotton plant (*Gossypium* spp.), may be more subject to disturbance of the normal flowering and fruiting processes (shedding of blossoms or young fruits, etc.) by such factors as change in the water supply or the ratios between the different elements of plant food in the soil. In addition to its apparent regulatory action on the internal mechanism controlling hydration, it is to be expected that the duration of the light period will tend to modify the external evaporative forces involved in transpiration. It seems possible that this may be a factor of importance in the action of the long days of summer in checking the growth of certain plants, although no definite evidence on the subject is available.

Regardless of its actual causative significance, there seems to be no doubt that hydration is subject to definite regulation by length of day and, hence, change in the degree of hydration is definitely correlated with change in form of expression in the plant. Apparently the optimal light period for apogeotropic growth also is optimal for maximum hydration. Progressive loss of power to fully utilize carbohydrate and other nutrient material in accomplishing increase in stature seems to be correlated with progressive loss of capacity to maintain the transpiration stream. Development of sexually produced as well as vegetative reproductive structures, tuberization, and the general phenomena of dormancy seem to follow as natural sequels.

CONCLUSION

In this paper considerable data are presented which tend to demonstrate the importance of the seasonal range in length of day as a factor in initiating and regulating sexual reproduction in plants, thus confirming the results of investigations reported in an earlier article. Experiments are described, also, which bear on certain quantitative aspects of this response. Results of fairly extensive investigations on the relation of length of day to the formation of tubers and bulbs are presented in some detail. Observations, largely of a preliminary nature, are reported on length of day as a factor in various other responses of the plant, including character and extent of branching, root growth, formation of pigment, abscission and leaf fall, dormancy, and rejuvenescence. Apogeotropic growth or increase in stature as affected by the daily light

period is given somewhat detailed consideration in its apparent relation to flowering and fruiting and the other responses mentioned.

It appears that the duration of the daily illumination period not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material. The evidence available at this time indicates that for each species there is an optimal light period for maximum upward elongation of the stem or increase in height. For some species this optimal light period is furnished by the longest days of summer in the temperate zone while for other species the intermediate length of day of spring and fall is optimal. In the latter group of plants the excessive length of day of midsummer probably initiates flowering, and in the case of some woody perennials the formation of resting vegetative buds. Certain species, moreover, are caused to die back, form bulbs, and enter into a midsummer rest period.

In the first-named group of species progressive shortening of the daily light period initiates a series of responses, including flowering and fruiting, tuberization, character and extent of branching, dormancy, all of which are prominent features in annual periodicity. Reducing the light period below the optimal for stem elongation by a certain definite decrement tends to divert the plant's activities more or less quantitatively toward sexual reproduction. There seems to be an optimal light period, therefore, for flowering and fruiting. Exposure to this optimal light period commonly induces rapid senescence and death, as typified in annuals, probably as a result of the intense flowering and fruiting. With day lengths ranging between the optimal for growth and the optimal for sexual reproduction the tendency toward division of the plant's energies between these two types of activity is manifested in the everblooming or everbearing condition, shifting of the quantitative relationship between relative vegetative and reproductive activity, change in the size of the individual fruit or seed, delayed and sparse flowering and fruiting, cleistogamy, and other abnormalities. Moreover, in the natural seasonal change in length of day there is considerable difference between the effects of the change away from the vegetative optimum toward the flowering and fruiting optimum and those resulting from the change in the opposite direction. Data of interest have been presented, also, on the time required for imparting the stimulus or condition for flowering by appropriate change in the light period.

Further reduction of the light period by a sufficient decrement below the optimal for sexual reproduction tends to induce intense tuberization, a feature marking the final stages in reduction of stem elongation. There is a more or less clearly defined optimum for tuberization. Here, again, with a light period ranging between the optimal for tuberization and the optimal for flowering there is a tendency toward division of the energies of the plant between these two features of development. Under these conditions sexual reproduction and tuberization may proceed simultaneously, a division of activity which is commonly seen in herbaceous perennials. Tuberization as a result of decrease in the daily light period involving deposition of carbohydrate in relatively condensed or dehydrated forms indicates marked loss of power to utilize the product of photosynthesis in elongating the stem or in developing flower and fruit. It is most typically associated with the stemless or rosette form of foliage development, representing, perhaps, maximum divergence between income and effective utilization of the photosynthetic product for promotion of growth.

One of the characteristic effects of change in the light period from optimum to suboptimum for stem elongation is to promote branching. Thus, the apical bud loses its dominance and lateral buds become active. As stages in the response to progressive departure of the light period away from the optimum for elongation of the axis may be noted increased branching at the top, middle, or base of the aerial portion of the axis; various degrees in erectness of the resulting branches; change from aerial to underground type of stem and change downward in direction of growth of these underground stems.

The decreasing length of day of fall is an important factor in causing perennials to enter upon the winter period of dormancy. Abscission and leaf fall, also, appear to be induced by this shortening of the light period. Abrupt and sufficiently pronounced change from long-day to short-day conditions, however, tends to cause deciduous woody perennials to behave like evergreens in retaining the foliage leaves, and this tendency also is discernible even in certain typical annuals. Furthermore, the light period, through quantitative features of its action on the formation of vegetative resting and reproductive structures, may affect to some extent the conditions of emergence from dormancy.

During the winter months the relation between the length of the daily light period and the temperature becomes especially important. Because of the short days prevailing in this period the income of the plant through photosynthesis is reduced to the minimum and growth conditions are otherwise unfavorable, so that high temperatures are likely to establish an unfavorable ratio of income to outgo and thus may easily prove disastrous to the plant.

It seems probable that the annual cycle of length of day, affording as it does the only consistently rhythmic feature of the external environment, is a dominant causal factor in phenomena of plant periodicity which have been regarded by many as explainable only on the basis of an "internal rhythm." This view is strengthened by the fact that definite periodicity in plant development is observable under various climatic conditions, from the equatorial regions to the poles, while the only consistently rhythmic factor of the environment applicable throughout this range is the length of day. In arriving at a correct estimate of the significance of the annual cycle in length of day in regulating periodic plant responses it must be understood that full expression of periodicity is conditioned on the specific requirements of the particular plants in question as to temperature and other environmental factors. The internal mechanism concerned in the marked regulatory action of the length of day on plant growth has not been fully determined, but there is strong evidence that in some way the degree of hydration of the living cell content is brought under very delicate control by the ratio of the number of hours of sunlight to the number of hours of darkness in the 24-hour period.

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PLATE 1

Poinsettia:

The individuals in center and at right are the same plants that are shown in earlier stages in Plate 19, A. Early in September the plant shown at left in Plate 19, A, was transferred to the greenhouse, where it received electric illumination from sunset till midnight in addition to the natural daylight. In response to the lengthened daily light period, the high-colored bracts soon began to assume a green color, particularly in transmitted light. Some of the bracts lost the original red color more or less completely. Finally, a new shoot appeared at the apex of the flowering stem. In the early stages of development this shoot was of the weak, pendent type and the bark of the stem was red. Subsequent development of this stem is shown in Plate 19, B. The plant at right, maintained under long-day conditions throughout the test, continued to develop a vegetative stem. The plant at left received the same treatment as the individual in center, except that the electric illumination from sunset till midnight was omitted. Nothing remains of the inflorescence except the naked stem. Painted from a photograph made January 9.

BREUNER & KESSLER CO. PHILA

B

A

PLATE 2

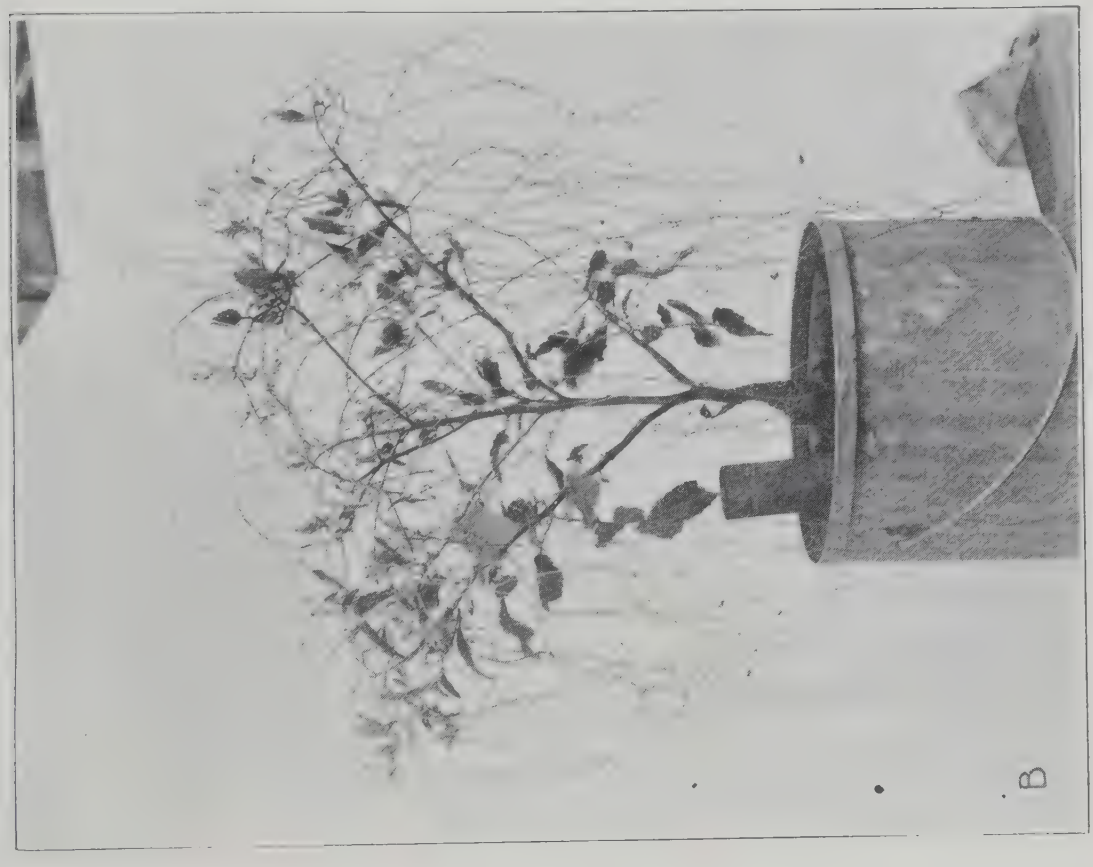
A.—*Helianthus angustifolius* L. Plants at right were exposed to the full daylight period supplemented with electric light from sunset till midnight, throughout the test which began January 27. Plants at left were similarly treated till September 2, when they were given a 10-hour daily light exposure. First blossoms appeared on these plants October 4. It is seen that the short-day conditions induced very free flowering and the response was prompt. Photographed October 15.

B.—Morning-glory (*Ipomoea hederacea* Jacq.). Plants from seed which germinated June 11. The first blossom on plant at left, exposed to a 10-hour day from the outset, was open July 10. The first open blossom on plant at right, exposed to the natural length of day, appeared September 9. Photographed July 13.

PLATE 3

A.—Scarlet Globe radish plants, from seeds which germinated May 19. These plants were prevented from flowering by exposure to a day length of only seven hours till October 20, when they were transferred to the greenhouse and allowed to receive the full natural period of daily illumination during the months of November, December and January. By February 1, one of the plants showed evidence of elongating a flowering stem in response to the increasing length of day, and the daily light period was again shortened to seven hours. Elongation of the stem was soon slowed down. It is seen from the photograph that branching is confined chiefly to the upper portion of the stem. The first blossom appeared March 20. Photographed April 17.

B.—Final stages of development of the radish plant shown in A, at right. It is seen that the type of development has been markedly changed by continued exposure to seven hours of light daily. Progressive weakening in power to elongate the primary axis is accompanied by increased transfer of activity to the lower branches. Thus, the effect is to change the general contour of the plant to conform more nearly with that of many woody perennials. It will be observed that the weak, pendent stems which eventually appeared, grew to unusual lengths. These are typical effects of a light period which is markedly suboptimal for increase in stature. Photographed June 2.


B

A

A

B

PLATE 4

A.—Apple seedlings. Plants at right were exposed to 10 hours of light daily during the summer months; those on left received the full daylight exposure of summer. It is apparent that growth is more rapid and vigorous with only 10 hours of light daily than with the 14 to 15 hours of daylight of summer in the latitude of Washington. This would indicate a tendency toward an increased rate of growth with decrease in latitude.

B.—Seedlings of *Acer negundo*. Beginning May 2, the plants at left were exposed to a 10-hour day while the plants at right were exposed to the full day length of summer. It is obvious that in contrast with the apple this species grows vigorously under long-day conditions and could not flourish under the length of day prevailing at Washington during the winter months. Photographed September 22.

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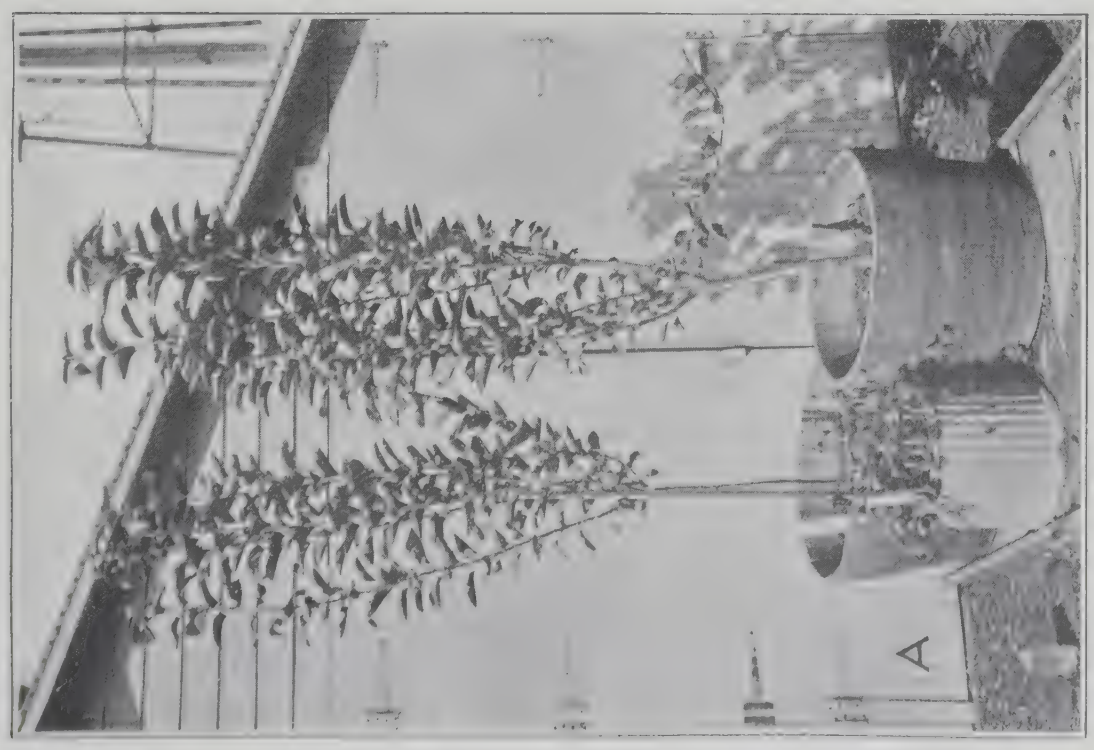
PLATE 5

A.—Grafts of the Baldwin apple, transplanted May 2 and exposed to 12 hours of light daily, beginning May 17. Plant at left is a standard Baldwin while plant at right is a Baldwin graft on dwarf (Paradise) stock. The trees in this series grew vigorously throughout the summer. Only one individual of the control series exposed to the full length of day survived the transplanting. This individual grew very slowly till August, but thereafter there was a marked increase in rate of growth. Photographed September 28.

B.—Goldenrod (*Solidago juncea*). Plant at left exposed to electric illumination during the winter months from sunset till midnight, in addition to the natural daylight. Plants at center and at right exposed to natural daylight only. As indications of initial formation of flowering stems induced by the increasing length of day became apparent on March 10, these plants thereafter were allowed to receive only 7½ hours of light daily. Stem elongation was quickly checked and only the leaf-rosette type of activity was continued.

B


A

A

B

PLATE 6

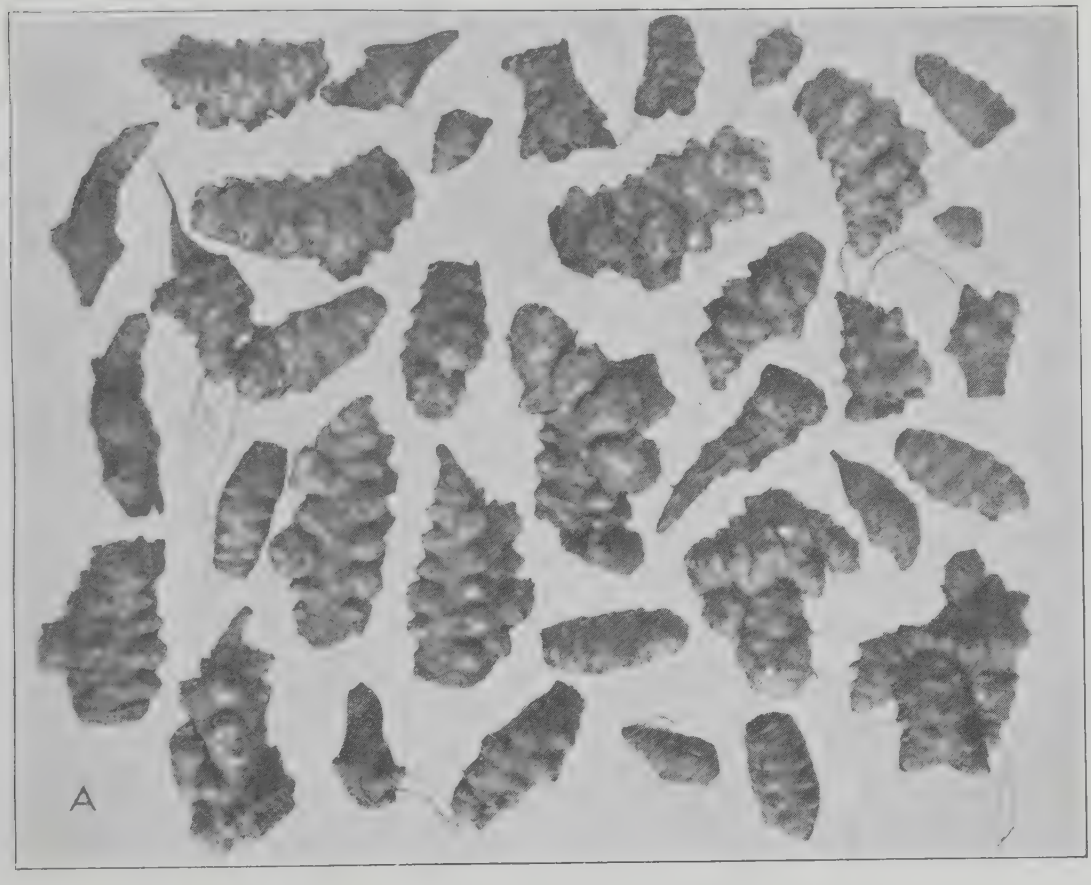
A.—Scarlet Runner bean (*Phaseolus multiflorus*). The plants in this series were exposed to the full length of day during the summer and on October 15 were transferred to the greenhouse where they received electric illumination from sunset till midnight, in addition to the natural daylight. Under these conditions there was no tuberization of stem or roots. Photographed January 18.

B.—Scarlet Runner bean plants which were exposed to a 10-hour day till October 15, when they were transferred to the greenhouse and allowed to receive the full natural daylight period of late fall and early winter. Under these short-day conditions there was marked tuberization of the roots while the growth of vines was considerably less than under the long-day conditions. Photographed January 18.

PLATE 7

A.—Tubers of Jerusalem artichoke. The mother plants were grown from tubers which germinated April 25 and were exposed to a 10-hour day from the outset. Although flower buds were formed, they were unable to open. By August 15 the tops of the plants were dead. Tuberization of the underground stems was complete, that is, there was no elongation of unthickened stems. Note the numerous unusually prominent buds on the tubers. Apparently the downward translocation of nutrient materials was so marked as to cut off the requisite food supply of the flower buds. Photographed October 1.

B.—Tubers from control planting of artichoke exposed to the full seasonal length of day. These plants began flowering September 22 and were in full bloom October 1. The difference in tuber formation between these plants and those under a 10-hour day is striking. It is readily seen that under the longer daily light period there was marked elongation of the underground stems and only partial tuberization. The number of new units formed was greater than under the 10-hour day, but they were much smaller in size and showed only inconspicuous buds. In this case the activities of the plant approach more nearly an even distribution between sexual reproduction and tuber formation. Photographed October 1.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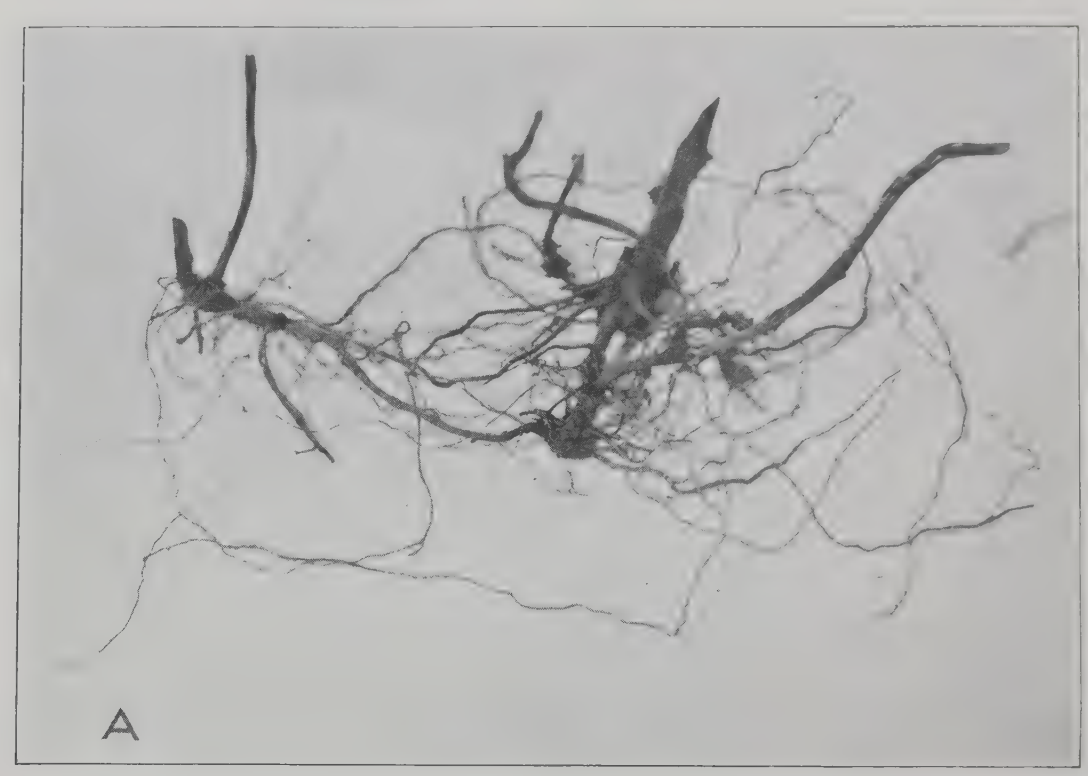
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PLATE 8

A.—Underground portion of Irish potato plant of the McCormick variety. This plant was grown in the greenhouse during the summer months and received electric illumination from sunset till midnight in addition to the natural daylight. No tubers were formed, and while flower buds appeared they were unable to open. Instead of becoming tuberized some of the buds borne on the underground stems at once germinated and formed offsets, thus eliminating the usual rest period. The aerial stems of the mother plant reached unusual heights. As the length of day was progressively reduced there was corresponding increase in tuber formation (see text, page 891).

B.—Plants of table beet, roots of which had been stored in an outdoor pit during the previous winter. Plants at left were exposed to the full seasonal length of day, beginning April 1, and began flowering in June. Plants at right, exposed to a 10-hour day began the development of flowering stems but were unable to flower, the apex of the stem being transformed into a leaf rosette. The branches and leaves are more numerous than under the longer day. Photographed June 14.

PLATE 9

A.—Silverskin onion plants from sets which were planted May 19. Plant at extreme right is representative of a series exposed to the full seasonal length of day in the open air. These plants flowered in July, formed bulbs, and passed through the usual summer rest period after the tops had died down. In the series represented by the second plant from the right, which were exposed to a 13-hour day, the growth of tops was much greater while the bulb was delayed in forming and was reduced in size. The plant in center is representative of those exposed to a 10-hour day. In this case there is no summer rest period and no bulb is formed, the tops remaining green indefinitely. The individual at extreme left shows the behavior of plants grown under the natural length of day in the greenhouse, where the temperature was considerably higher than out of doors. The behavior was the same as that of the controls in the open except that the size attained was materially reduced. With the addition of electric illumination from sunset till midnight in the greenhouse only a slight attenuating effect was observed, as shown by the second plant from the left. Photographed July 28. (Compare Pl. 18, A.)

B.—Biloxi soybeans, showing partial tuberization of the stems in the vicinity of the nodes which results from exposure to a length of day which is too short to admit of free flowering and fruiting. Under these conditions flower and fruit are unable to fully utilize nutritive material reaching them, and these materials are deposited in the surrounding tissues in the more condensed form characteristic of tuberized structures.

B

A

A

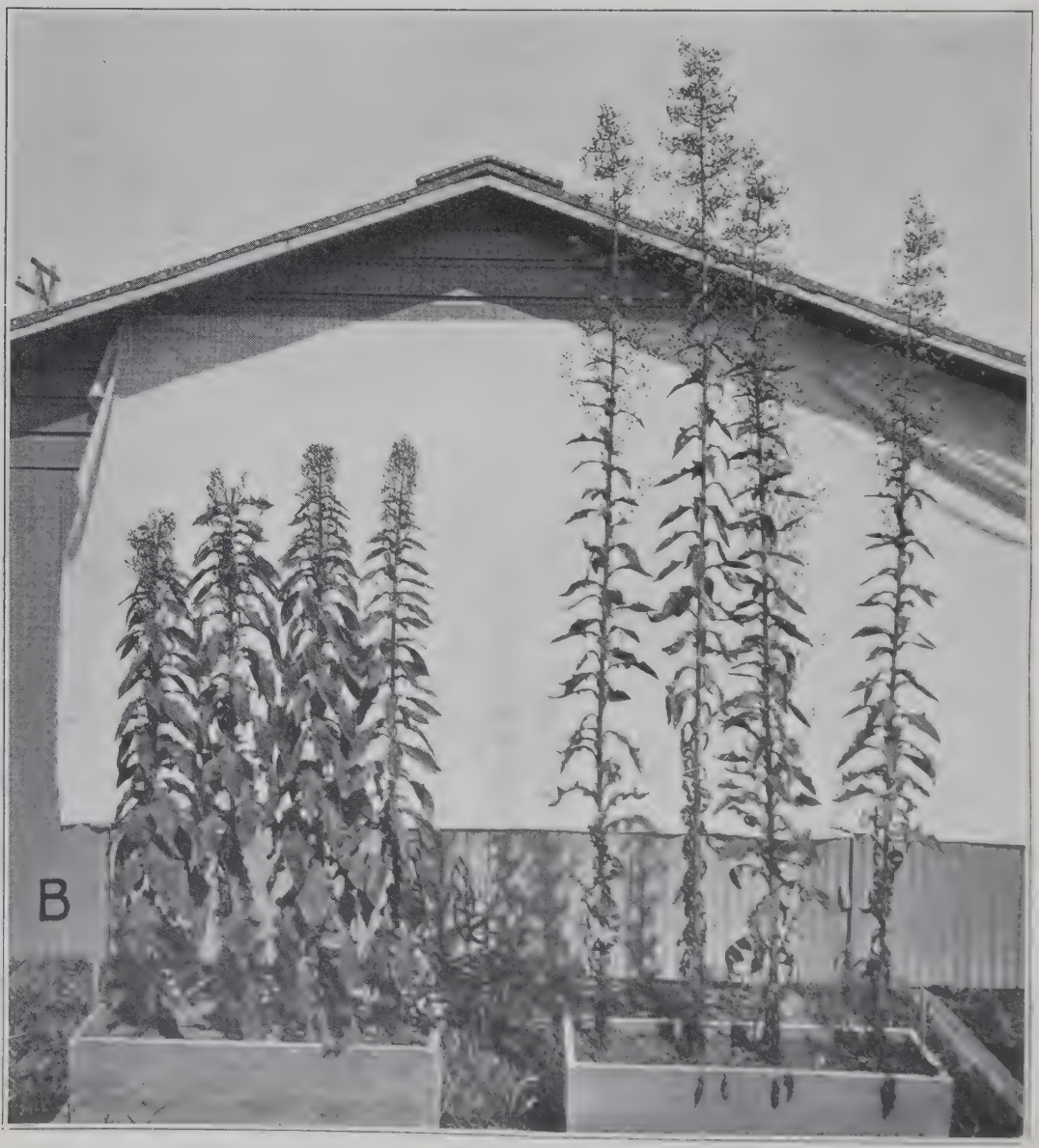
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PLATE 10

A.—Yam (*Dioscorea alata*) from the tropics. Tubers at right are from plants grown under a 12-hour day, beginning May 14, while the tubers at left are from control plants exposed to the full seasonal length of day. The equatorial length of day favors intense tuberization, while the long summer days of high latitudes causes marked development of fibrous roots, with very limited tuberization. The basal flattening of one of the large tubers is the result of "spreading" over the floor of the container in which the plants were grown, suggesting a sort of gravitational settling somewhat like that, for example, of fresh putty. This seems to represent a rather extreme case of loss of capacity for apogeotropic functioning. Photographed October 7.

B.—Wild lettuce (*Lactuca spicata*). Plants at left, exposed to a 10-hour day beginning March 29, produced much larger, coarser leaves than the control plants (at right) exposed to the full summer length of day. Under the short-day exposure both leaf and stem were far more hairy than under the long-day conditions. The stature was greatly reduced, but the time of flowering was not affected.

PLATE 11

A.—Honey sorghum, grown in greenhouse and exposed to electric illumination from sunset till midnight during the summer and fall months. Under these conditions branching was confined mostly to the top of the plant. This type of branching resembles that seen in Cosmos at time of flowering and represents an advance toward the sterile, nonbranching type of stem, as compared with basal branching under a shorter length of day. Photographed November 4.

B.—Control planting or sorghum exposed to natural illumination only In this case there is extensive branching at the base, the new shoots developing rapidly and flowering freely.

A

B

A

B

PLATE 12

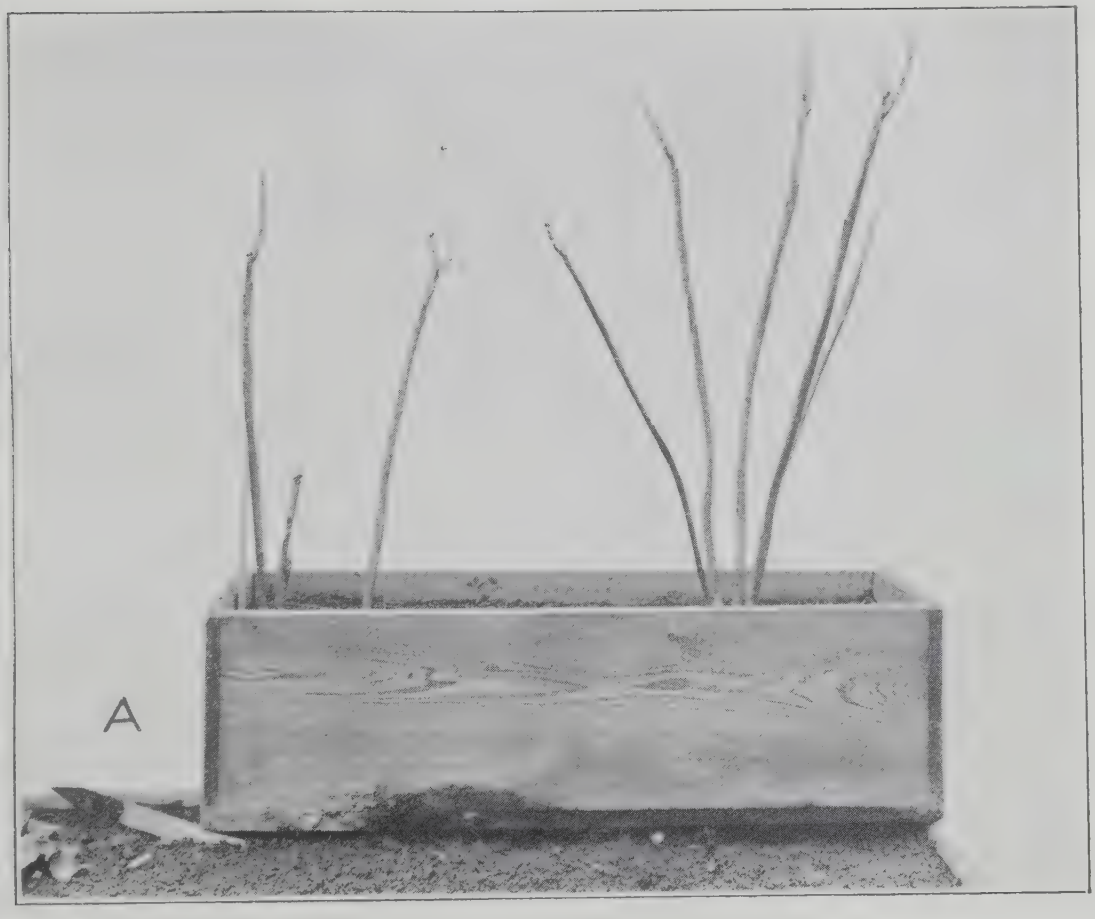
A.—*Oenothera biennis*. Plants at left were exposed to the full daylight period of the spring months. The individual at right received only 10 hours of illumination, beginning March 19. It is seen that the short-day conditions caused a decided weakening in power to elongate the primary axis, resulting in development of numerous basal shoots. In this case dominance of the apical bud is lost, a characteristic response to a suboptimal light period for elongation of the stem. Photographed June 21.

B.—Sorrel (*Rumex acetosella*). Plants at left received electric illumination from sunset till midnight, in addition to natural daylight in the greenhouse, during the winter months. These plants developed numerous aerial flowering stems and in general showed the typical summer behavior of this species. The plants at right, which received only natural illumination, were unable to develop aerial stems but formed thick rosettes of leaves. In reality, however, these latter plants were more active than appearances indicate, for a very large number of underground stems were developed. This behavior of sorrel furnishes further demonstration of the fact that the position, character, and extent of branching are subject to regulation by the length of day. The leaf-rosette form of activity during the fall, winter, and early spring is typical of many plants. Photographed March 25.

PLATE 13

A.—Sumac (*Rhus glabra*). These trees were transplanted in April and remained out of doors till September 8, when they were transferred to the greenhouse. They received the full seasonal length of day throughout the test. The leaves were shed at about the usual time, and the plants remained dormant till spring. Photographed January 11.

B.—Sumac, treated in all respects like the trees in A except that when transferred to the greenhouse in September the trees received electric illumination from sunset till midnight. The leaves were retained through the winter, and, while growth of the original stems was checked, a number of new shoots appeared from the roots about January 1. Photographed January 11.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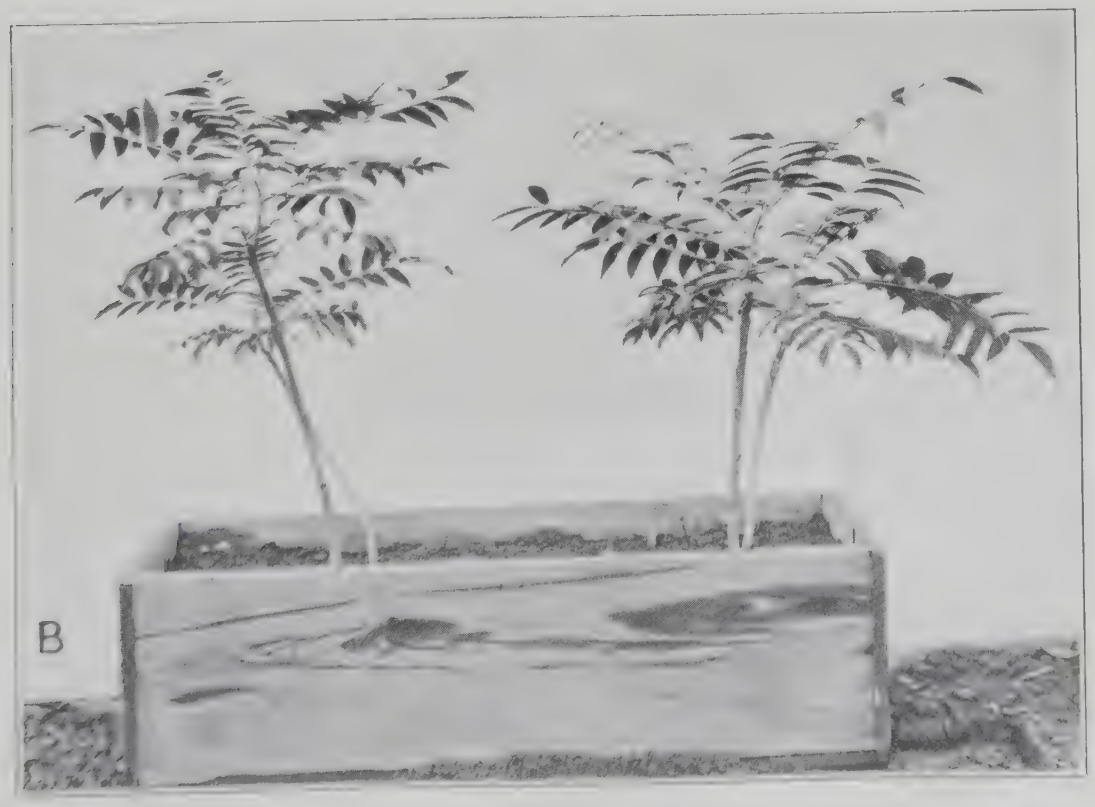
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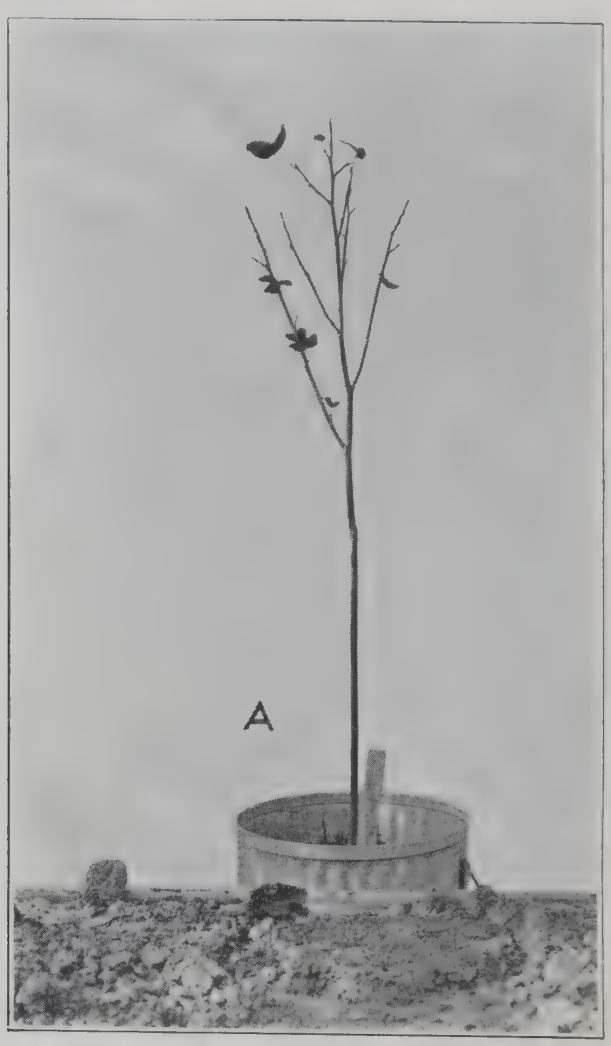
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PLATE 14

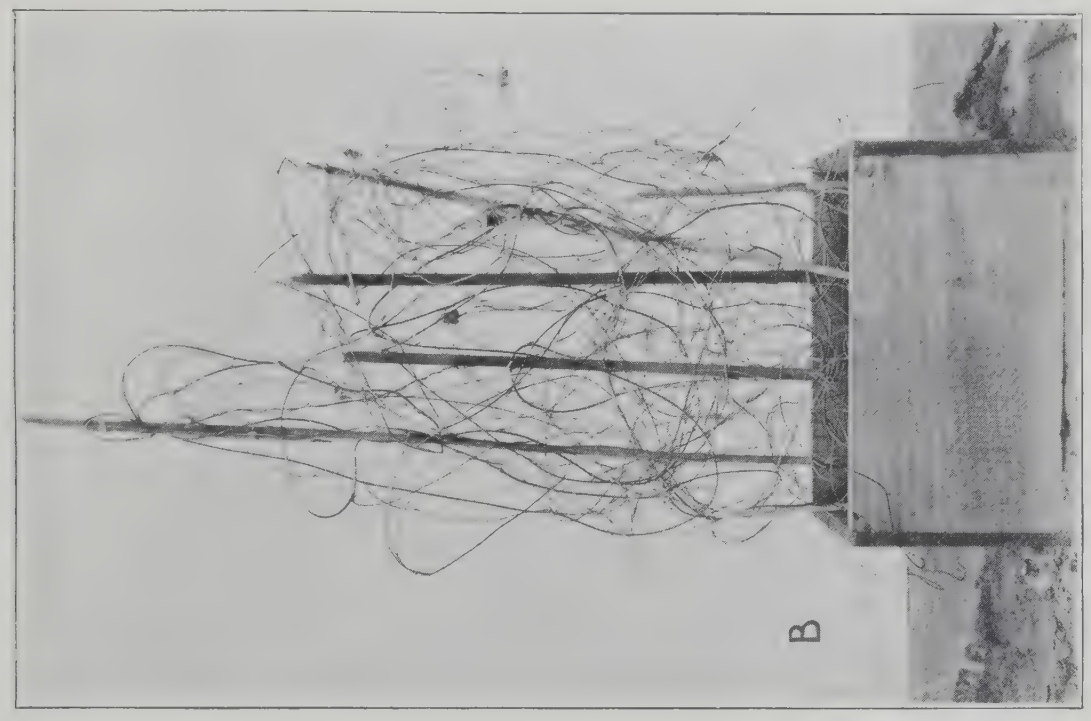
A.—Tulip tree (*Liriodendron tulipifera*), transplanted in April and placed out of doors. On September 8 the tree was transferred to the greenhouse. There was the usual loss of leaves by abscission, followed by dormancy which continued through the winter. Photographed November 18.

B.—Tulip trees, treated in all particulars like the individual shown in A except that after transfer to the greenhouse the trees received electric illumination from sunset till midnight, in addition to the natural daylight. Under these conditions, the rest period was eliminated. Growth was promptly renewed and continued throughout the winter. A characteristic feature was failure to form the absciss layer when old leaves died here and there on the trees. These dead leaves remained firmly attached to the stem, in marked contrast with the usual behavior of this species. Photographed November 18.

PLATE 15

A.—Kudzu (*Pueraria hirsuta* (Thunb.) Scheid.). Seedling which was exposed to a 10-hour day during the summer months. Under these conditions there was little top growth but the basal portion of the stem was moderately tuberized. The plant was transferred to the greenhouse September 15, where it gradually passed into a condition of dormancy which seemed to be fully reached by December 1. The rest period, however, was short and by January 11 new shoots appeared. These shoots grew vigorously, although the leaves were of a darker green than is usual under a longer daily light period. Photographed February 19.

B.—Control seedling of Kudzu treated in all respects like the plant shown in A, except that it was exposed to the full seasonal length of day during the test. In the greenhouse the plant finally lost all leaves and became dormant like the plant in A. Emergence from dormancy was delayed as compared with plant in A, and new shoots did not appear till February 16. Photographed February 19.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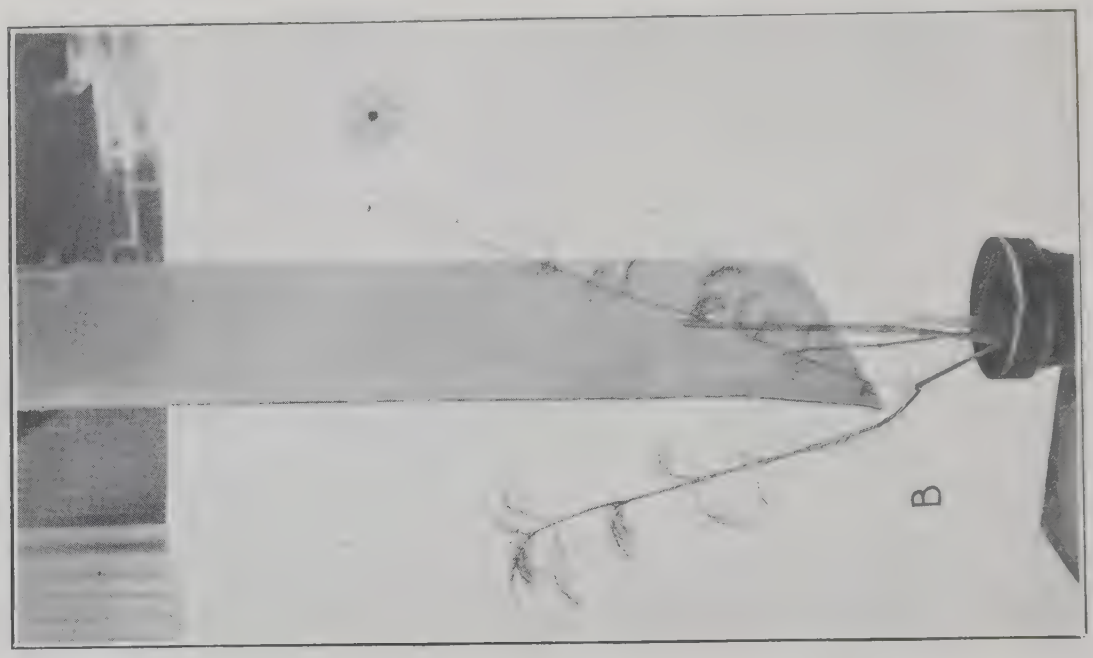
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PLATE 16

A.—*Bidens frondosa.* Seedling planted in greenhouse; germinated December 23. Beginning January 30, when flowering had begun, the plant received electric illumination from sunset till midnight, in addition to the natural daylight. The total original growth of the plant attained under natural illumination, including ripened fruits, is seen in front of the cardboard. As is shown, this original stem growth died, but new shoots appeared, and these reached giant proportions under the influence of the long daily illumination period. Thus, rejuvenescence was effected by use of a favorable light period. This plant may be regarded as embodying two different types of development at different periods in response to change in the duration of the daily light period. Photographed June 21.

B.—Cosmos. The branch at right flowered promptly in response to the short winter day to which it was exposed, and the usual evidences of advancing senility followed in due course. The branch at left of screen remained sterile and continued to grow in response to the added electric illumination from sunset till midnight. The dominant influence of this branch over the flowering branch in its effect on basal portions of the plant is evident. In this instance two different types of development take place simultaneously on the same plant in response to difference in the light period (compare with A). Photographed March 25.

PLATE 17

A.—Stems of Cosmos, grown in winter in greenhouse. Electric illumination was used from sunset till midnight as a supplement to the natural daylight. Sections of stems in center and at right show the effects of cutting back in causing local swelling of the nodes, followed by development of new branches. This behavior indicates a strong upward flow of sap from below, resulting from exposure to a length of day favorable to elongation of the vegetative stem. This behavior is not observed when the plant is exposed to short-day conditions. The section of stem at left illustrates the condition of the nodes at the time cutting back was done.

B.—*Hibiscus moscheutos*. Seedlings of previous summer's growth which remained out of doors during the winter. Beginning March 27, the two plants at left were given a 10-hour day while the control plants at right continued to receive the full seasonal length of day. Under the 10-hour day small, weak basal shoots finally appeared in May, as seen in front of the cardboard. These shoots were unable to grow and soon perished. New shoots appeared on the controls early in April, and these continued to develop normally. Photographed June 21.

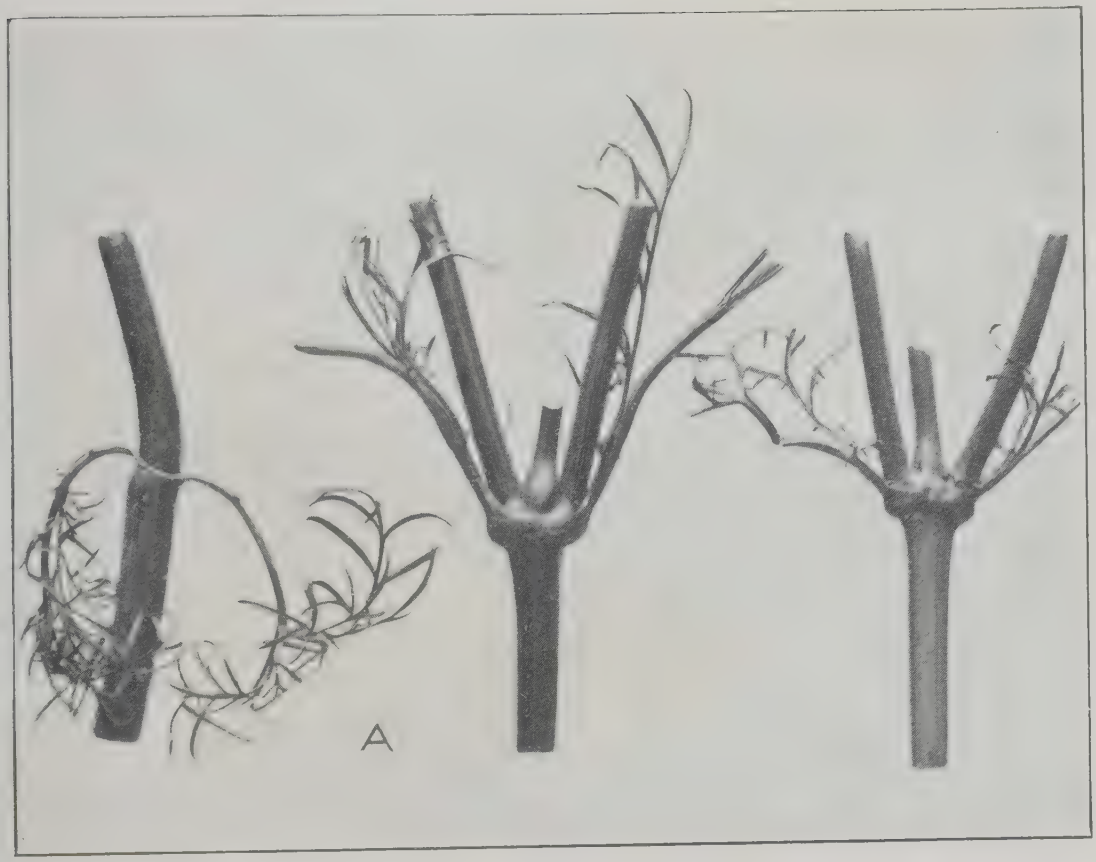
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PLATE 18

A.—Silverskin onions, grown with different conditions of light intensity and with differences in the length of the daily light period. The plants at left were exposed to full intensity of the sunlight for the entire day. Plants at right were exposed to sunlight for the entire day, but the intensity of the light was reduced to a third of the normal by means of a cheesecloth shade. The plants in the center were exposed to the full intensity of the sunlight, but the duration of the daily period of illumination was reduced to 10 hours (5.30 a. m. to 3.30 p. m.). It is easily seen that the strong reduction in intensity of the light has had but little effect on bulb formation and entrance into the summer rest period. Shortening the length of day by less than a third (and thus reducing the total radiant energy of the sunlight received by a far less proportion), however, has effectively prevented the formation of bulbs and the appearance of the summer rest period.

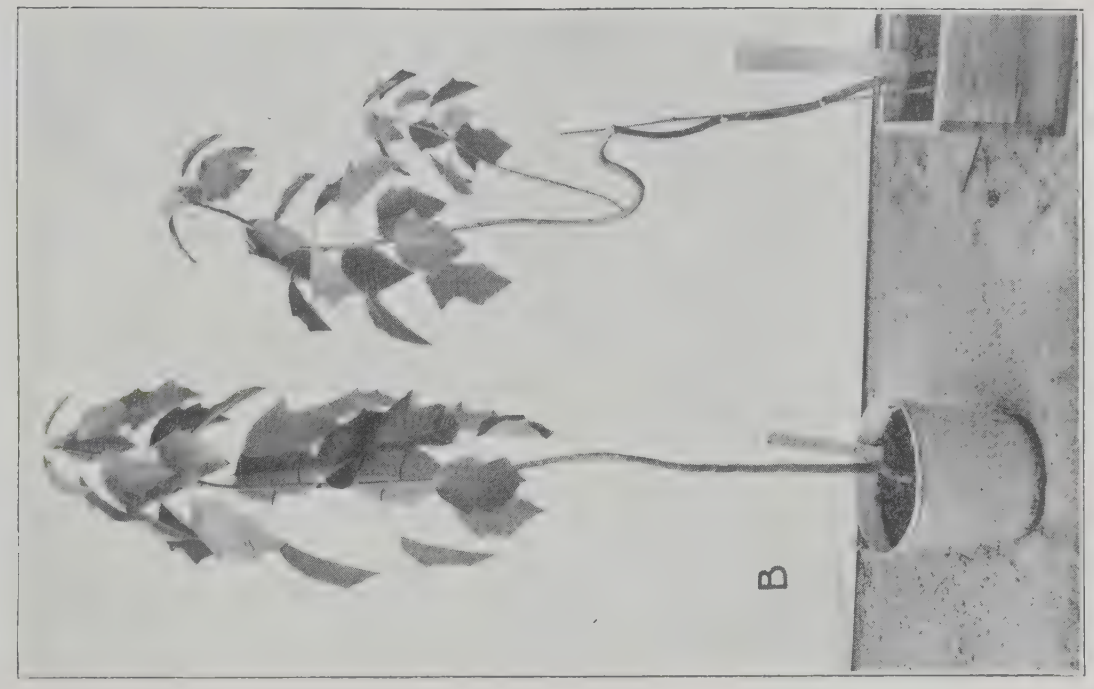
B.—Violet. Plant at left in flower shows the characteristic behavior under the relatively short days of spring and fall. The plants in the center and at the right were exposed to electric light from sunset till midnight as a supplement to winter daylight. Beginning January 30, however, the daily light period of the center plant was reduced to 7½ hours. In a few days there was partial loss of turgidity in the leaf stems, resulting in the prostrate condition to be noted in the photograph. Subsequently the characteristic blue blossoms of spring appeared in this plant. This experiment seems to suggest that permeability of the plasmic membrane or the degree of dydration of the protoplasm is affected by the length of day. This prostrate type of development is a characteristic feature in the development of many plants under short-day conditions. Photographed April 17.

PLATE 19

Poinsettia:

A.—Beginning July 9, the plant at left was exposed to a 10-hour day. Before the close of August this plant had flowered and was richly colored. The control plant at right, exposed to the seasonal length of day, of course, did not flower till early winter. This experiment is of interest in showing that the green chlorophyll pigments of the leaf may give way to other pigments with appropriate change in the light period. Photograph made September 13.

B.—In the plant at right can be seen the completed rejuvenescence effected in the weak, pendent shoot which appeared at the tip of the stem bearing an inflorescence, as shown in Plate 1. This rejuvenescence was accomplished by exposure to a daily illumination of about 18 hours, after flowering had been induced by exposure to a 10-hour day as shown in Plate 19, A. The U-shaped portion of the woody stem resulted from the pendent position of the new shoot as it first appeared. The short horizontal section of stem is that on which the inflorescence was borne. Plant at left illustrates the tall, spirelike development of the stem, absence of branching, and progressive loss of lower leaves which yield their plastic nutrient materials to the upper plant parts, all of which are typical features of the response to a long day. Photographed July 13.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AGAMERMIS DECAUDATA COBB, STEINER, AND CHRISTIE; A NEMA PARASITE OF GRASSHOPPERS AND OTHER INSECTS.[1]

By N. A. COBB, *Agricultural Technologist in Charge;* G. STEINER, *Nematologist and Technologist,* and J. R. CHRISTIE, *Assistant Nematalogist, Office of Agricultural Technology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

This is a preliminary account of a joint investigation carried on at Washington, D. C., and Falls Church, Va., and relates to a nema *Agamermis decaudata*, parasitic in insects, particularly in Orthoptera. The work at Falls Church is carried on with the cooperation of the Bureau of Entomology at its eastern field station, and all the insects examined have been determined by officers of that bureau. Other valuable assistance has been rendered by approximately 150 American zoologists and entomologists, whose aid has made possible a first tentative map of distribution of the parasite in the United States.

The general oversight of the work has been handled by the senior author, who has given special attention also to methods and apparatus, and to the structure and behavior of the free-living larva. Dr. Steiner gave special attention to the structure and taxonomy of the parasitic and post-parasitic stages. He has also handled nearly entirely the difficult matter of the literature and nomenclature. Prof. Christie has given special attention to field and laboratory work connected with the hosts and their habits, the effect on the host, the habits and distribution of the post-parasitic forms in the soil under natural conditions, and to the rearing of both host and parasite.

In the present account it is not proposed to do more than sketch some of the main features of the work, which has involved thousands of dissections, hundreds of carefully executed laboratory and rearing experiments, and the invention and trial of dozens of different pieces of special apparatus adapted to various features of the work.

A broad numerical basis has been given the facts disclosed, since the work has for its definite object the economic application of the data secured. The summary presents two aspects, the latter of which is subdivided: First, the results show positively that, in certain cases believed to be typical, the nema *Agamermis decaudata*, is an important factor in the birth rate of certain injurious insects; second, the application of this fact may be exploited in two ways, (*a*) by colonization—that is, moving the parasite from places where it exists to places where it does not exist, or is rare; the experiments show that this can be done, either by transferring diseased insects, such as grasshoppers, or by transferring the parasite itself in one form or another; (*b*) by forecast—since methods of detecting the parasite have been developed to a point where it is believed possible to hazard some form of prediction. Just as, if we can not control the weather, it is at least important to be able to predict it, so, if we can not prevent the ravages of an insect, it is at least important to be able to predict when the ravages are likely to occur. In this respect the results of the investigations are considered positive.

[1] Accepted for publication Mar. 7, 1923.

SOURCES AND DETERMINATION OF THE NEMAS

In the main the material for these investigations was collected at Falls Church, Va., but questionnaires were sent to zoologists all over the United States. Nearly 160 of these were returned, some with collected Mermithid material, and many with very interesting information about distribution, hosts, etc.

The examination of this contributed material is not yet finished, and therefore no more than a somewhat brief sketch of the American Mermithid land-fauna can be given, together with some aspects of the economic value of the family.

On leaving the insect host the nemas enter the soil. There have been observed thus far a dozen or more species of Mermithids from American soil, mostly new. The two most common are what have been called *Mermis albicans* Von Siebold and *M. nigrescens* Dujardin, which are among the first Mermithids made known and also the ones most frequently mentioned in literature. Both apparently have a wide geographical distribution.

The determination of Mermithid species is difficult; one reason is that often only larval forms are obtainable, and another is that Mermithids do not possess obvious morphological characteristics. The present studies were much aided by the excellent papers of Meissner (*5*, *6*),[2] Rauther (*7*), and Hagmeier (*3*). The latter emphasized the opinion that among the principal and best aids in classifying Mermithids are the sense organs on the head, since they rarely change during the life of the individual.

Whereas *Mermis nigrescens* seems to show little variation and to be represented in this country by the typical European form, the American *Agamermis decaudata* is unusually difficult of determination, even for a Mermithid, because it presents an extensive series of variations, between the extremes of which there is a complete gradation of intermediate forms. These varieties, or races, and apparent hybrids are best recognized by the structure and arrangement of the head sense organs. As far as can be judged, the European *albicans* as described by Meissner (*5*, *6*), Rauther (*7*), and Hagmeier (*3*) has not yet been found in this country. The chaos of these forms is probably greatly augmented by hybridization. Cases occurred at Falls Church where the mating female and four males (four males with one female) all differed in the structure of the head sense organs.

Another interesting evidence of the existence of a number of different races in *Agamermis decaudata* is the fact that almost any collection that includes a number of female specimens will show intersexes—that is, females with more or less well developed male sexual organs. Experimental biology is showing that such intersexes may be frequent in the "artificial" crosses of closely related forms, races, or genotypes. *A. decaudata* as it occurs in nature, is not a clean-cut species, but rather a mixture of races and their hybrids.

The origin of the races of *Agamermis decaudata* is still uncertain. Perhaps the great variety of hosts may have some influence.

It may be considered questionable whether there are *decaudata* races special to certain hosts. We do not know yet, but it appears as if larvae of various races are entering hosts without special choice.

[2] Reference is made by number (italic ‘to "Literature cited," p. 926.

A review of the information obtained from the returned questionnaires also emphasizes the economic value of Mermithids, a fact surmised earlier by a number of entomologists but never proved in a substantial way. Of 130 returned questionnaires, 53.6 per cent state that Orthoptera were the hosts. In 51 cases the writers made observations regarding the effect of the parasite, mostly on an individual host. Of these latter observers, 85 per cent report an injurious effect on the host, 71 per cent stating that the normal development of the host was prevented, 47 per cent stating the fecundity of the host was reduced, and 53 per cent that the host was killed. Most of these statements seem mainly the result of fortuitous observation on a limited scale; in general, they corroborate those already mentioned in the literature. Hagmeier (*3*) estimated that on a meadow 33 per cent of all grasshoppers were infested with Mermis; Glaser and Wilcox (*2*) in this country estimated infestation up to 60 per cent; Leidy (*4*), very frequent; Steiner's observations (*8, p. 223*) showed in a garden heavily infested with the snail *Limax agrestis*, 20 to 30 per cent of them mermithized.

EXPERIMENTS AT THE EASTERN FIELD STATION OF THE BUREAU OF ENTOMOLOGY AT FALLS CHURCH, VA.

During the early part of July, 1922, it was noticed that grasshoppers in the vicinity of Falls Church, Va., were infested by a Mermithid parasite. From that time until cold weather, collections were made over an area of several square miles, and a total of 3,332 grasshoppers was examined. Of the 824 Tittigonidae included in this number, very nearly all were *Orchelimum vulgare* Harris. Of the remaining 2,508 Acrididae, about 90 per cent were *Melanoplus femur-rubrum* Deg. Mermithid infestation was found to average 12 per cent, increasing to about 25 per cent in heavily infested regions.

Records based on the examination of 2,498 grasshoppers, the sexes of which were determined before dissection, show the males and females to be about equally parasitized.

As the nemas do not reach sexual maturity in the host, but moult once in the soil after leaving the host, the determination of the material taken from grasshoppers is a difficult task. However, it is certain that the greater number of the nemas were varieties or races of *Agamermis decaudata*, with an occasional species of another kind.

The parasites begin to leave the hosts by the latter part of August, if not before, and are nearly all out by the first of October. Their method of exit is by forcing their way through the body wall of the insect. In one carefully observed case the parasite issued from the side of the abdomen, head foremost, and was free in a very few minutes. The parasite was assisted by the kicking of the grasshopper, whose feet became entangled in the coils of the nema.

Once free from the host, the Mermithids make their way into the soil to a depth of 3 to 20 cm. The females go down from 4 to 8 cm., seldom deeper. Here they coil up in the "nest," forming a "knot," and apparently never again move about. The males move through the soil more freely, as it is evidently their function to seek out the females. The "knots" as dug from the soil during the winter contain one female, rarely more, and from one to seven males.

In order to determine the approximate Mermithid population in an infested region, 16 square feet of soil were examined to a depth of 30 cm. (12 in.). The yield was 132 Mermithids, or at the rate of 359,370 per acre.

Eggs are laid in large numbers until both the nemas and the wall of the nest become plastered with them. Egg counts show that each female lays, on an average, at least 5,000 eggs. Under the 16 square feet referred to in the preceding paragraph, 32 "knots" were surrounded with eggs. If the average number of eggs per nest is 5,000 (probably a very conservative figure) there will be, provided all the eggs hatch, 435,600,000 larvae per acre. Each foot will therefore support a population of 10,000, and each square inch of surface will have below it about 70 of these young Mermithids

The egg has the shape of a slightly flattened sphere, and measures 119 by 105 microns. The rate of its development is influenced by external conditions, particularly temperature. At the end of five or six days, with average September weather, the embryo takes the form of a single, circular coil, almost filling the shell. At this point in its development there is a rapid proliferation of cells at the posterior extremity, the ends of the loop overlap and in a short time several coils are developed. As the length of the embryo increases new coils are formed until, at the end of about a month, the young larva reaches its full length of a dozen or more coils—that is, 3 to 4 mm. The development of eggs deposited late in the fall seems to be retarded by cold weather, for material collected in March still contains the earlier stages of development. The hatching of the eggs is probably brought about by the warm spring weather, as eggs brought into the laboratory in winter and kept at room temperature hatch freely after three or four days.

After hatching, the larvae work their way to the surface of the soil and enter the newly hatched grasshopper nymphs.

Artificially infested grasshoppers containing six or eight Mermithids die in about eight days. In fact it may be doubtful if grasshoppers harboring more than one parasite ever reach maturity. The ovaries of infested females which do survive are vestigial, never producing functional eggs, and it is likely that the males are also rendered sterile. The exit of the nemas, sometimes at least, results in the death of the grasshopper, and there is every reason to believe that this is always the case.

STRUCTURE AND BEHAVIOR OF THE FREE-LIVING LARVA

The rear five-sixths of the *decaudata* larva has a certain uniformity of structure. Here the cuticle and body wall are typically nemic. The body cavity is filled by a moniliform series of elements, having somewhat the general character of nemic intestinal cells, but there is no lumen and no trace of an anus. Each of these 50 to 70 cells (trophocytes), in addition to its nucleus, protoplasmic network, and minute proteid granules, is packed with spherical 3–4 micron fat globules, melting at about 55° C., and contains a thin-walled catabolic vesicle holding a birefringent crystal. As the larva ages, the fat disappears, while the vesicle increases, thus pushing all other contents of the cell to one end. In a few weeks no fat remains, and the nema has a segmented aspect. By expending the fat of the trophocytes, the posterior five-sixths of the nema becomes the active mechanism for driving the strongly cephalated anterior sixth to, and into, the host.

On entering the host the driving mechanism is shed at a predetermined, elaborately prepared node, and only the cephalic portion becomes parasitic. The node at which this automatic amputation takes place is prominent in the free-living larva. Persisting as a little altered terminal scar, it indicates that no moult occurs during the parasitic life. Usually

the driving mechanism is left outside the host, but young larvae in which the node is unripe may take the driving mechanism inside, where, in the course of a few days, it is shed. The nema requires from two to five minutes to enter the host. This period is often preceded by a few minutes of waiting—perhaps devoted to some internal preparation for the onslaught. Entrance is bored at any thin part of the cuticle of the very young larva of the host—namely, on the head, thorax, abdomen, or legs.

The cephalic end of the free-living larva is highly specialized. No particular reference is made here to its acute, hollow, protrusile spear, or its six inconspicuous cephalic papillae, amphids, nerve-ring, oesophageal lumen, and longitudinal chords,[3] most of which closely resemble those of the "*Mermis albicans*" of Hagmeier. The bifurcated, symmetrical, two-celled renette lies in the neck behind the nerve-ring. The oesophagus ends posteriorly in a short, small, cylindroid swelling, which at intervals of about a second may exhibit the sudden peristaltic motions characteristic of the swallowing act of nemas.

The intestine comprises two sections, of which the posterior is about two bodywidths long. The four times longer anterior section, comprising sixteen cells, has a distinct, narrow, refractive, tubular lumen, and is flanked by three unequal glandular structures having their free ends caudad, the largest of which, the right, extends throughout the length of this section of the intestine, while the two others—equal, narrower, and on the left—have only half that length. These organs are packed with minute dodecahedral, colorless, in the main nonstaining (carmine, osmic), refractive granules, of which those in the largest organ are very much the largest; beside this, there are physiological differences, in that the two smaller organs stain differently intra vitam from the large one and behave differently after the larva enters its host. During the early days of parasitism these three organs disappear, so they evidently have to do with the physiological changes connected with the inception of taking in food from the host. The intestinal lumen in the 20–30-celled posterior section is of a different, nonrefractive character (wider and surrounded by a very thin nonrefractive wall), and this section presents the elements commonly seen in the intestinal cells of free-living nemas.

The sexual blastomeres nearly always lie in the axil between the two portions of the intestine.

After entering the host the posterior portion of the intestine changes and gives rise to a syncytiumlike (?) structure of relatively enormous size. As the three glandular organs disappear, the anterior 16-celled section of the intestine also begins a relatively huge growth, at first (20 days) forming a somewhat moniliform series of 16 organs (urocytes?) with large nuclei very like the four organs of *Tetradonema* (1). The early growth of the two portions of the intestine is not so very unequal, so that the sexual anlage comes to lie nearer the middle of the body. Along with this enormous growth of the intestinal elements the longitudinal chords,[3] especially the lateral, increase greatly in volume from back to front.

In spite of the fact that the free-living *decaudata* larvae will live for hours or even days on thawing ice, when placed in water or moist earth at 32° F. they are much injured by the congealment. Two or three such experiences kill them. One exposure to zero Fahrenheit kills them. It seems probable that they can not survive even light frosts in nature.

[3] Hitherto usually referred to in the literature as the "longitudinal fields."

These free-living larvae can exist in soil for several months, during which time experiments have shown them to be apogeotropic and positively phototropic, rheotropic, pedotropic,[4] and xenotropic.[5] Within the limits of the vernal temperatures of arable soils in subtemperate regions, the larvae are positively thermotropic. Once dried they die.

From what has been previously said concerning the deposition of the eggs, it will be seen that these tropisms tend to bring the larva to the surface of the ground and into contact with its host.

The entrance of the larva into its host, although very easily brought about in the laboratory, has not been observed in nature and doubtless will be a matter very difficult of observation. The forecast is that the larvae will come to the surface of the ground in late spring, leave the soil, lurk among the decayed and living vegetation near the surface of the ground, and enter the host mostly at night.

The bobtailed parasitic larva, 25 by 700 microns, grows at a rapid rate, often increasing a millionfold in two to three months.

BRIEF GENERIC AND SPECIFIC DIAGNOSES[6]

Agamermis Cobb, Steiner, and Christie n. gen.

Mermithidae with terminal mouth, no mouth papillae, 6 head papillae, vagina S-shaped, 2 spicula; parasitic larva decaudate. Moults once in the egg.

Agamermis decaudata Cobb, Steiner, and Christie n. sp.

Characters as given on pages 924–926 herein, and as shown by Hagmeier's figures (except fig. 14) for his *Mermis albicans* (*3*). Amphids, however, varying somewhat from those figured by Hagmeier; no cervical papillae seen. Moults once in the egg.

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[4] πέδον = soil.
[5] ξένος = host.
[6] Full descriptions will be published later.

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SUMMER IRRIGATION OF PIMA COTTON[1]

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INTRODUCTION

Summer irrigation problems need to be distinguished from those of the spring months. In the spring the problem is chiefly one of controlling the growth of the young plants and their habits of branching so as to avoid excessive vegetative development. After these early stages are passed, and the plants are flowering and fruiting, the question is how often to apply water to the best advantage for the development of a maximum crop. The answer will depend upon differences in soil and other conditions that affect water requirements, but treatment can be adjusted to the needs of the crop, as indicated by the behavior of the plants.

A first experiment with summer irrigation, recognized as a distinct problem, was made with Pima[2] cotton at the Cooperative Testing Station,[3] Sacaton, Ariz., in 1920,[4] and yielded some interesting and entirely unexpected results. The plan was to compare the behavior of plants, to which different frequencies of irrigation had been applied, after they had reached a normal early fruiting stage. In a series of such comparisons the results were alike, with no such differences of growth as have been observed in many experiments that included earlier stages of plant development. The general indication is to confirm and emphasize the importance of controlling the early development of cotton in order to bring the plants to a normal early fruiting stage. When this condition is established summer irrigation problems are simplified.

RECOGNITION OF A NORMAL FRUITING STAGE

Early in July, when the experimental treatment began, the plants were about 18 inches high and had from five to eight fruiting branches, were mostly without vegetative branches, and just beginning to flower. This form of plant is recognized as the early fruiting stage of a normal development. The plants had been brought to this condition by withholding water in the early growing period and by delaying thinning until the plants were from 10 to 14 inches high. This was done in order to check any tendency of the plants to grow rank, or put out an excessive number

[1] Accepted for publication July 2, 1921.

[2] Pima is a variety of Egyptian cotton bred and developed by the Department of Agriculture and is grown extensively in the Salt River Valley of Arizona and the Imperial and San Joaquin Valleys of California.

[3] The Cooperative Testing Station is located in the Gila River Valley at the Indian village of Sacaton and is approximately 30 miles southeast of Phoenix, Ariz. The soil and climatic conditions are similar in many respects to those in the Salt River Valley.

[4] An effort was made to repeat the experiment in 1921 on the same plots with as nearly as possible the same treatments, but spring conditions were very unfavorable so that regular stands were not secured and rains in July and August interfered with the control of growth by irrigation, so that comparable results were not obtained.

of vegetative branches. It so happened that no irrigation was required until July when the treatments began. Copious winter rains and cold spring weather may possibly explain why water was not needed earlier.

Previous experiments have demonstrated that the most desirable and productive type of plant is one which utilizes to the fullest extent in fruiting development the space apportioned to it, without injury to its neighbors. Such plants have no vegetative branches, or only one or two, depending on spacing. To grow plants of this type it is necessary to observe precautions in thinning and avoid any unnecessary irrigation when the plants are young and easily forced into undesirable vegetative growth. When precautions are not taken the plants may develop several vegetative branches and become so crowded that only a late crop can be produced.

It is essential that the present experiment should not be confused with others that have been published, that dealt with the early development and control of the branching habits of the plants.[5] The treatment cotton receives before fruiting begins undoubtedly is reflected in all of the subsequent behavior of the plants and is a factor in determining the water requirements during the fruiting period. The objects and conditions of experiments in the spring differ from those of the summer; hence the treatments are distinct problems.

CLIMATIC CONDITIONS

As the weather during the cotton-growing season is an important factor in judging the results of experimental data, a summary of the records is given.

The season of 1920, though unusual in many respects, was more favorable for a reliable consideration of several cultural features than could be had in a normal year. Lack of heavy summer rains made the irrigation problems more definite, and an unusually cool spring assisted materially in controlling the early growth of the plants. On the other hand, the maximum air temperatures in July were unusually high, providing a test of the effects of hot weather.

Table I gives the monthly average maximum and minimum temperatures from March to October, inclusive, for the years 1910 to 1919, the 10-year average for each month, and the monthly averages for 1920. In comparing the average monthly maximum and minimum temperatures for 1920 with the averages for the corresponding month in the preceding years it will be seen that those for 1920 generally are lower, and, with the exception of the July maxima, are all below the 10-year average.

The first killing frost occurred on November 29, but there had been light frosts, which did little damage, as early as October 14. For a period of 10 consecutive years prior to 1920 the average date of the first killing frost at Sacaton is November 17.

The summer rains, which usually occur in the latter part of July and the early part of August and are a complicating factor in the problem of irrigation of cotton, were almost negligible in the season of 1920, as shown in Table II. This precipitation has not been considered, since the showers were so slight and so widely separated that the plants were not affected, but the data are presented in Table III.

[5] COOK, O. F. A NEW SYSTEM OF COTTON CULTURE AND ITS APPLICATION. U. S. Dept. Agr. Farmers' Bul. 601, 12 p., 2 fig. 1914.

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TABLE I.—*Average monthly maximum and minimum temperatures from March to October, inclusive, Sacaton, Ariz., from 1910 to 1919*

AVERAGE MAXIMUM TEMPERATURE (DEGREES FAHRENHEIT)

Year.	March.	April.	May.	June.	July.	August.	September.	October.
1910	87.3	89.9	99.2	104.9	106.1	104.5	103.5	91.3
1911	83.7	86.2	94.0	103.1	99.3	102.8	98.7	85.7
1912	72.1	77.9	91.9	104.1	101.3	100.2	97.7	84.5
1913	73.2	87.8	93.8	100.0	103.1	104.3	100.0	87.5
1914	80.0	86.9	96.0	99.7	103.0	107.0	100.0	84.9
1915	75.5	84.3	85.6	103.9	105.1	106.0	100.4	95.5
1916	84.0	87.4	95.5	105.9	105.7	103.4	99.7	86.2
1917	73.7	81.6	86.7	103.6	102.5	99.9	95.6	91.3
1918	75.1	82.4	87.5	101.9	100.0	97.1	97.9	87.1
1919	70.5	83.7	90.4	100.5	97.6	99.5	92.9	80.6
Av. of 10 years	77.5	84.8	92.0	102.8	102.4	102.5	98.6	87.5
1920	71.4	78.9	91.0	99.0	104.5	99.0	95.3	82.6

AVERAGE MINIMUM TEMPERATURE (DEGREES FAHRENHEIT)

Year.	March.	April.	May.	June.	July.	August.	September.	October.
1910	40.4	50.4	57.3	65.4	73.5	74.8	70.2	52.3
1911	46.4	48.7	52.5	64.6	72.7	76.2	65.8	52.3
1912	44.3	46.3	55.6	64.9	69.5	70.8	58.5	53.4
1913	38.7	49.0	56.0	62.7	70.6	73.1	64.2	48.5
1914	44.2	48.5	56.3	70.2	75.0	76.0	65.0	53.9
1915	39.1	49.4	52.1	63.6	70.0	72.0	62.6	49.8
1916	45.6	50.6	52.6	63.1	73.3	72.9	66.2	49.1
1917	36.7	46.6	55.6	62.6	75.9	69.2	68.0	54.4
1918	47.5	47.6	53.0	70.6	73.2	69.1	65.1	52.5
1919	38.4	48.5	57.4	63.2	74.0	72.4	66.8	49.6
Av. of 10 years	42.1	48.6	54.8	65.1	72.8	72.7	65.2	51.6
1920	41.2	44.3	53.2	60.4	70.7	70.1	61.3	48.6

TABLE II.—*Monthly precipitation, in inches, at Sacaton, Ariz., for an 8-month period from 1910 to 1919 compared with that for the year 1920*

Year.	March.	April.	May.	June.	July.	August.	September.	October.
1910	0.38	Trace.	.....	.....	0.65	1.45	.....	0.15
1911	.47	..do ..	.....	Trace.	4.11	.82	1.38	1.40
1912	2.61	.85	.38	.98	4.27	1.02	.....	.96
1913	.....	.40	.....	.92	.92	.73	.04	.18
1914	1.02	.16	.10	.34	3.25	1.77	.30	2.28
1915	1.01	1.16	.69	.10	2.44	1.05	.....	.....
1916	.71	.45	.....	.....	1.19	.63	3.61	.02
1917	.40	.11	1.29	.....	3.33	.05	.43	.....
1918	1.17	.19	.....	.20	1.49	1.92	.....	.29
1919	.55	.20	.04	Trace.	3.76	1.63	1.80	.15
Average	.83	.35	.25	.25	2.54	1.11	.75	.54
1920	1.42	.03	.17	.23	.48	.89	.21	1.21

TABLE III.—*Daily precipitation, in inches, Sacaton, Ariz., from March to October, inclusive, 1920*

Mar.	1	Trace.	July	13	.35
	10	0.47		21	.11
	23	.07		22	.02
	24	.53	Aug.	2	.28
	25	Trace.		10	.09
	27	.34		14	.01
Apr.	15	Trace.		19	.24
	19	.03		25	.27
May	21	.17	Sept.	3	.05
June	26	.13		14	.16
	27	.10	Oct.	20	.86
				31	.35

ARRANGEMENT, PLANTING, AND THINNING

The soil of the experimental plot is a black sandy loam, very well suited to the growing of cotton. Clear well water is used for irrigation, which is distributed from a concrete ditch provided with leak-proof gates. Nine sixth-acre borders[6] were used for the experiment, arranged in series of three borders each. The first series, containing borders C1–3, C1–4, and C1–5, had been in alfalfa the previous year. The other two series, covering borders C1–9 to C1–14, inclusive, and forming a solid block, had grown cotton in the previous season. The soil of series I is slightly richer than that of the others.

The experiment was planted on March 29 and April 1 with a 2-row planter, using a 44-inch row spacing, and a good stand was obtained. Unfavorable low temperatures through April and early May kept the ground cold and retarded the growth of the young seedlings. During this cold period injuries from the "sore-shin" disease were not uncommon in the Salt River Valley, while at Sacaton, with lower temperatures prevailing, the damage from this source was considerably greater, but the stand was materially affected only in a few cases.

Series I was thinned on June 4, when the cotton was about 12 inches high, leaving the plants as nearly as possible a foot apart in the row. The plants in this series were in a slightly more advanced stage of development than those of the other two series. Series II and III were thinned on June 14, when the plants had reached the same stage of development as had been attained in series I, and with the same row spacing.

PLAN OF IRRIGATION

The irrigation treatments that were applied to the three borders in each series were recorded as "normal," "medium-heavy," and "heavy," respectively. An explanation of these terms is necessary in the absence of any such definite or recognized practices.

"Normal" irrigation was the application of water in such a manner as to keep the plants flowering heavily and with little or no wilting of the leaves in the middle of the day. This is considered the best cultural practice in the Salt River Valley.[7]

"Heavy" irrigation was to apply water at definite intervals regardless of the condition of the plants or soil. As near as possible a 10-day period

[6] Border is a term applied to a portion of a field used as an irrigation unit.

[7] HUDSON, E. W. GROWING EGYPTIAN COTTON IN THE SALT RIVER VALLEY, ARIZONA. U. S. Dept. Agr. Farmers' Bul. 577, 8 p. 1914.

was allowed between irrigations of these borders. This period was known to be much shorter than usual, as irrigation records of these borders for previous years showed that intervals of 20 days or more had been used with good results.

"Medium-heavy" irrigation was planned to be intermediate, as nearly as possible, between the "normal" and "heavy" treatments. These periods varied, of course, with the "normal" irrigations and ranged between 14 and 23 days, while the periods of the "normal" borders varied from 14 to 44 days.

In applying the water the borders were flooded to a depth of several inches, as uniformly as possible. In the absence of any means of measuring the amount applied, there were some fluctuations in the quantity a border received, but such differences were not considered sufficient to modify the results, in view of the generally uniform behavior of the cotton.

This plan of irrigation was adhered to in the months of July and August, during the period of maximum plant growth and the setting of most of the bolls. After the first of September the effects of irrigation were considered only in relation to the maturing of the crop, there being no advantage in further growth of the plants. Bolls that set later than September 10 at Sacaton, even if they reach mature size, are likely to be frozen and then not open.

APPLICATION OF WATER

All the borders were irrigated on July 2 for the first time after planting. At this time the plants were from 14 to 20 inches high and were just beginning to flower. The next application of water was on July 13 to the three "heavy" borders, although they showed no signs of needing more moisture. A week later, July 20, the "medium-heavy" borders were irrigated, before any indications of wilting could be detected. The "heavy" borders had their third irrigation on July 23, when they were still visibly wet and there was no practical need of more water.

About July 28 limited areas of plants in the "normal" borders in series II and III began to show wilting of the leaves in the middle of the day, although the plants were growing rapidly and not showing flowers at the top. With this indication of the need of water, these two plots were irrigated on July 30.

It was apparent from the behavior of the plants that the "normal" border of series I would not require irrigation as soon as the "normal" borders in series II and III. As a result, the irrigation of both the "normal" and "medium-heavy" borders of series I was later. For the "heavy" border of series I the 10-day interval of irrigation was maintained, as in the other series, until August 24, when this border was not watered because the plants showed unmistakable signs of distress by a yellowish green color and an almost complete cessation of growth, while the soil obviously was too wet.

After August 13, no further irrigations were required to mature the crop of the "normal" and "medium-heavy" borders in series I, but the corresponding borders of series II and III received two irrigations in September. The need of water in September was indicated by a more yellowish color of the foliage, although the leaves did not wilt in the cooler weather. The "heavy" borders in all three sections were irrigated once in September, but water was needed earlier in series I than in series II and III.

The irrigation dates for the borders of each series during the season are given in Table IV.

TABLE IV.—*Irrigation dates*

SERIES I, IN ALFALFA, 1919

"Normal;" C1-3.	"Medium-heavy," C1-4.	"Heavy," C1-5.
July 2	July 2	July 2
		13
	20	
		23
		Aug. 3
Aug. 13	Aug. 13	13
		Sept. 3

SERIES II AND III IN COTTON, 1919

"Normal," C1-9 and C1-12.	"Medium-heavy," C1-10 and C1-13.	"Heavy," C1-11 and C1-14.
July 2	July 2	July 2
		13
	20	
		23
		Aug. 3
30	Aug. 6	
		13
Aug. 20	20	
		24
Sept. 7	Sept. 7	
28	28	Sept. 28

A summary of the data in Table IV shows that the "normal" borders of series I received only two irrigations in July and August, as compared with three irrigations of the "normal" borders in series II and III. The "medium-heavy" borders were given three irrigations in series I during the same months and four irrigations in series II and III. The "heavy" border in series I had five irrigations in July and August, while in series II and III they received six.

METHODS OF RECORDING PLANT BEHAVIOR

As a means of keeping a record of the behavior of the plants during the fruiting season, general notes and comparisons of the condition of the plants in all the borders were made frequently. More detailed observations were made on the rates of growth, the numbers of flowers produced, and the shedding of young bolls. Such data were recorded from July 6 to September 15, inclusive, and were obtained from sections of rows containing 25 plants, selected early in the season to represent as fairly as possible the plants of the border.

In determining the rates of growth, the heights of the plants in the selected sections were measured carefully each week. The flowering notes were obtained by counting the number of flowers opening daily.

Shedding data were obtained by placing dated tags on all the flowers, the tags being fastened to the pedicels of the flowers so as to remain attached even if the flower or young boll was shed and fell to the ground. Each day when new flowers were being tagged, all of the young bolls which had shed were picked up and their flowering and shedding dates were recorded.

GROWTH OF THE PLANTS

During the early stages the plants developed rather slowly. The prevailing cool temperatures of April and May somewhat retarded the growth, and the plants averaged only 8 inches high in the latter part of May. An increased rate of growth was recorded in June, when the weather became very warm, but water was withheld in order to check any tendency for the plants to become rank or to produce vegetative branches. At the same time careful watch was kept to see that there was sufficient moisture available for a steady growth. The increase in height of the plants was approximately 10 inches during the month of

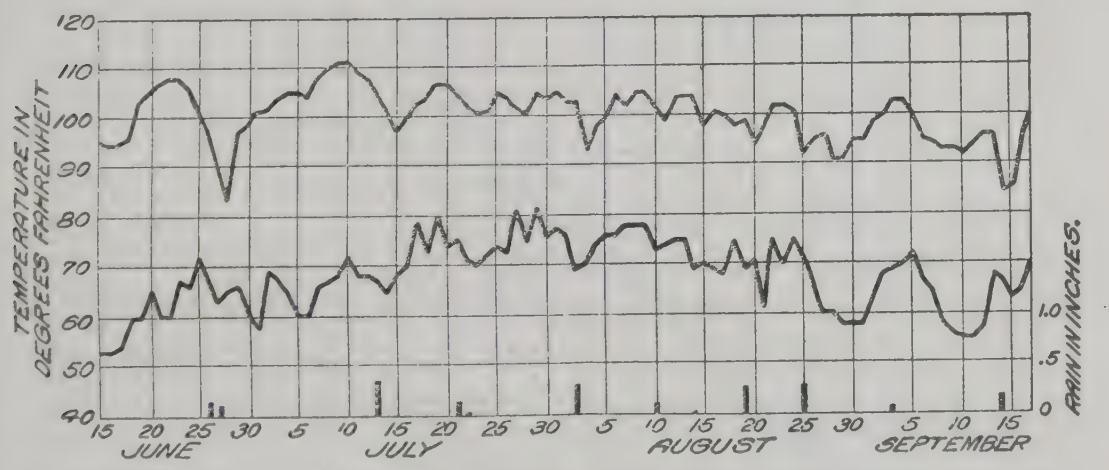

FIG. 1.—Daily maximum and minimum temperatures and precipitation at Sacaton from June 15 to September 15.

June. After the irrigation of July 2, when the experimental treatment began, the plants grew more rapidly but maintained a normal, fruitful habit.

Later development was marked by the uniformity of growth in all borders, notwithstanding the diversity of irrigation treatments. As shown in Table IV, which gives the average growth per week of 25 plants in each border and the average increase per week of the three borders that were treated alike, the development of the plants was at about the same rate throughout all the borders. It will also be seen that the plants in all the borders grew rapidly during July and early August, averaging an increase of 4.5 inches per week. During the week August 12 to 19 a decided decrease in the rate of growth was recorded in all borders, the plants growing an average of only 1 inch. A comparison of Table V with the climatological data presented in figure 1, shows there was no decided drop in temperature coincident with this retardation of plant growth, but during that week two small showers fell and there was general cloudiness of the weather. There is no reason to believe that moisture deficiency was the cause of the lower growth rate, as five of the nine borders had been irrigated on August 13, whereas the slower growth was recorded in all the borders. An increased rate of growth was obtained

during the following two weeks, although the mean temperature was slightly lower, but the weather was less cloudy with one rain on August 25. During the first week in September the growth became almost negligible, amounting to only 0.6 inch, and further records were not at-

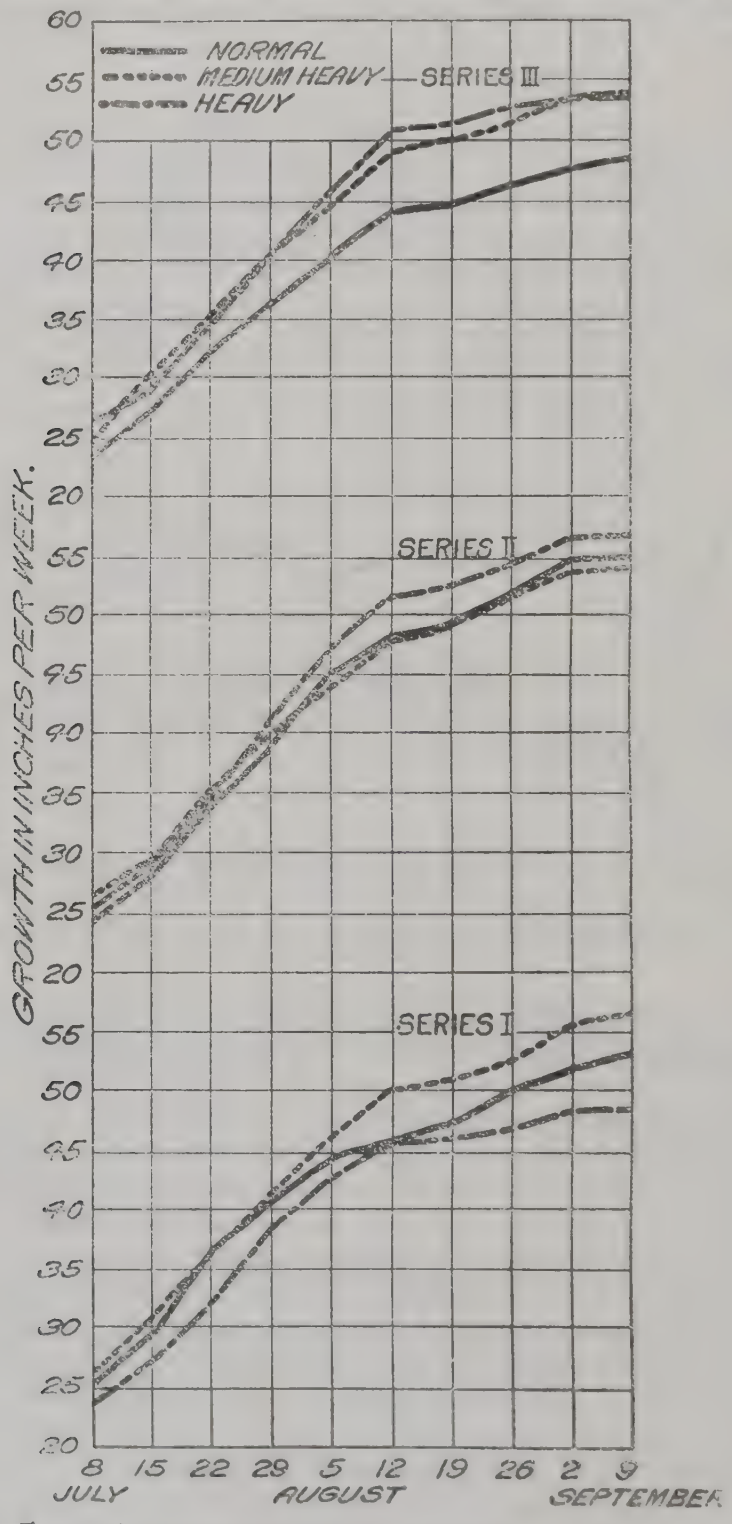

FIG. 2.—Average rate of growth for 25 plants in each border from July 8 to September 9.

tempted in view of the difficulty of detecting small differences in the height of the stalks.

A diagram showing the weekly rate of plant growth for the borders of each series is given in figure 2. It can be seen that at the end of the

growing season the average height that finally was reached by the "normal" borders (52.1 inches) was little different from that obtained in the "heavy" borders (52.9 inches) or in the "medium-heavy" borders (54.8 inches). There was no indication that the different amounts of moisture had affected the rate of growth, though it should be remembered that at no time was the amount of moisture allowed to get so low that the plants suffered noticeably, even in the "normal" borders that received the least water. The conclusion is that the additional or surplus water applied to the other borders was not utilized in making larger plants.

TABLE V.—*Average height in inches of 25 plants in each border by weeks from July 8 to September 9*

Date.	Normal irrigation.				Medium-heavy irrigation.				Heavy irrigation.			
	C1–3.	C1–9.	C1–12.	Average increase per week.	C1–4.	C1–10.	C1–13.	Average increase per week.	C1–5.	C1–11.	C1–14.	Average increase per week.
July 8	25.6	24.2	23.4	.....	26.1	26.3	24.2	.....	23.8	25.4	26.1	.....
15	29.9	28.0	27.6	4.1	30.9	29.4	30.3	4.7	27.1	28.7	29.1	3.2
22	36.5	34.0	32.6	5.8	36.4	35.1	35.4	5.4	32.0	34.3	34.8	5.4
29	40.6	38.7	36.3	4.2	41.6	39.9	40.3	5.0	38.2	41.0	40.3	6.1
Aug. 5	44.3	44.8	40.5	4.7	46.3	43.4	44.6	4.1	42.9	47.0	45.8	5.4
12	45.8	48.0	43.8	2.6	50.0	47.4	49.4	4.2	45.2	51.2	50.6	3.8
19	47.4	49.0	44.4	1.1	51.0	49.0	50.0	1.1	45.9	52.4	51.3	.8
26	50.0	51.5	46.3	2.4	52.5	51.4	51.7	1.9	46.6	54.0	52.6	1.3
Sept. 2	52.0	54.3	47.7	2.0	55.8	53.4	53.4	2.3	48.2	56.2	53.4	1.5
9	53.2	54.6	48.5	.8	56.8	54.0	53.8	.7	48.6	56.8	53.4	.3

Under some conditions the absence of any appreciable or consistent difference in the development of the plants in any of the treatments might be ascribed to lack of soil fertility. This possibility was considered, but the fact that in the previous year cotton has grown 6 to 7 feet tall on some of the land included in series II and III is thought to be sufficient evidence of an abundance of available plant food. Hence it is believed that the general uniformity of growth should be attributed to the stage of development the plants had attained before irrigation began rather than to the state of fertility of the soil or subsequent influences.

It is to be recognized, of course, that with other seasonal conditions, as hot weather in the spring or heavier rains in the summer, such uniformity of behavior might not have been maintained; but this does not make it less important to know how uniform the behavior may be under favorable conditions and how little difference was secured by such varied frequencies of irrigation as were included in this experiment.

DAILY PRODUCTION OF FLOWERS

The first flowers appeared early in July, and the record of flower production was started on July 6. At this time there were only 2 or 3 flowers opened per day on 25 plants, but the rate rapidly increased with the growth of the plants. The highest production was reached on July 31, with a maximum period extending for approximately 20 days, beginning about July 28 and ending about August 17. During this period an average of about 1½ flowers per plant per day was sustained. On September

15, when the flowering had almost ceased, there had been produced from 40 to 50 flowers per plant.

The daily flowering records of the 25-plant sections for each border are shown in Table VI. Although the results are in general harmony with the data of plant growth, this may not be apparent at first on account of the rather wide fluctuations, which no doubt are due in part to the limited numbers of plants included in the record. The general consistency of flowering behavior of the different treatments is more plainly shown by the superposed curves (fig. 3), which represent the totals of daily flower production of the three treatments, summarizing all the records of Table VI, and by the flowering curves of the three borders of series II (fig. 4).

By referring to Table VI, it will be seen that significant differences did not result from any of the three irrigation treatments, either in the number of flowers produced daily or in the total numbers of flowers

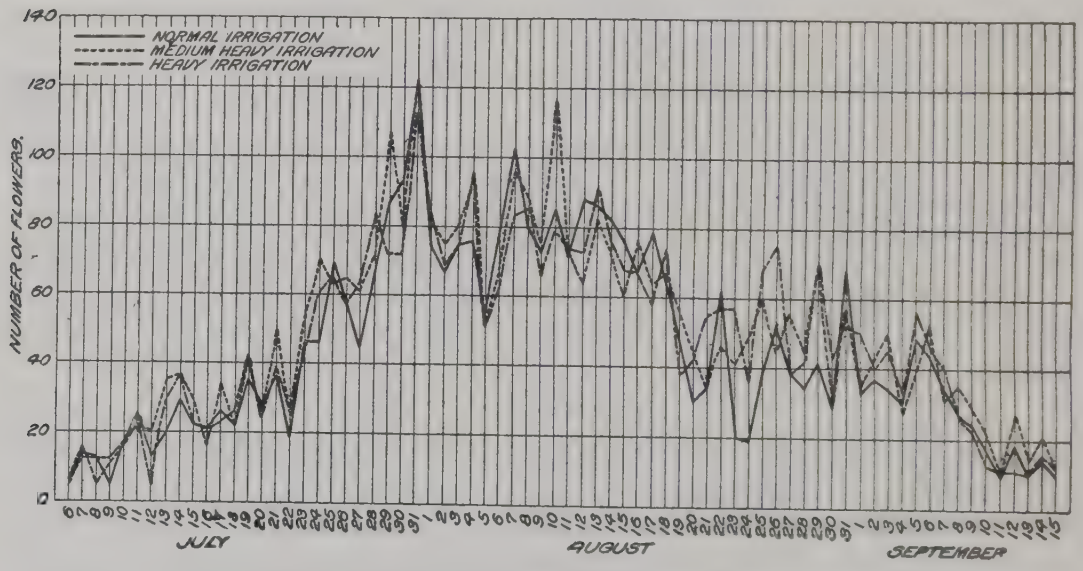

FIG. 3.—Total numbers of flowers per day on the three 25-plant sections of each treatment.

produced by each border for the season. This was due, no doubt, to the fact that the plants differed but little in size. That the total numbers of flowers produced on the 25-plant sections were not exactly proportionate to the numbers produced by each border as a whole can be seen by comparing these totals with the yield of seed cotton per border. The yields are the practical summaries, while the daily records serve as an index of the behavior of the plants.

A pronounced variation in the number of flowers opening from day to day often occurred in this experiment throughout the entire season, but independent of the irrigation treatment. Regardless of when or how often a border had been irrigated, there were days when all the borders had large numbers of flowers. Conversely, on days when only a few flowers opened the same tendency was generally apparent in all of the borders. This general coincidence in flowering becomes very striking when the flowers per day for the three borders treated alike are combined, giving the total flowers per day on 75 plants. From figure 3, it will be seen that the curves of the different treatments follow each other to a marked degree, although the range from day to day is often large.

Effort was made to correlate these fluctuations with temperatures and other factors, and some indications of correlation were found, but not enough to justify elaboration on the basis of such limited material. Until more definite knowledge is gained as to when the opening of a bud is determined, or what combinations of conditions cause the stimulus to be given, there is little basis for figuring correlations. The fluctua-

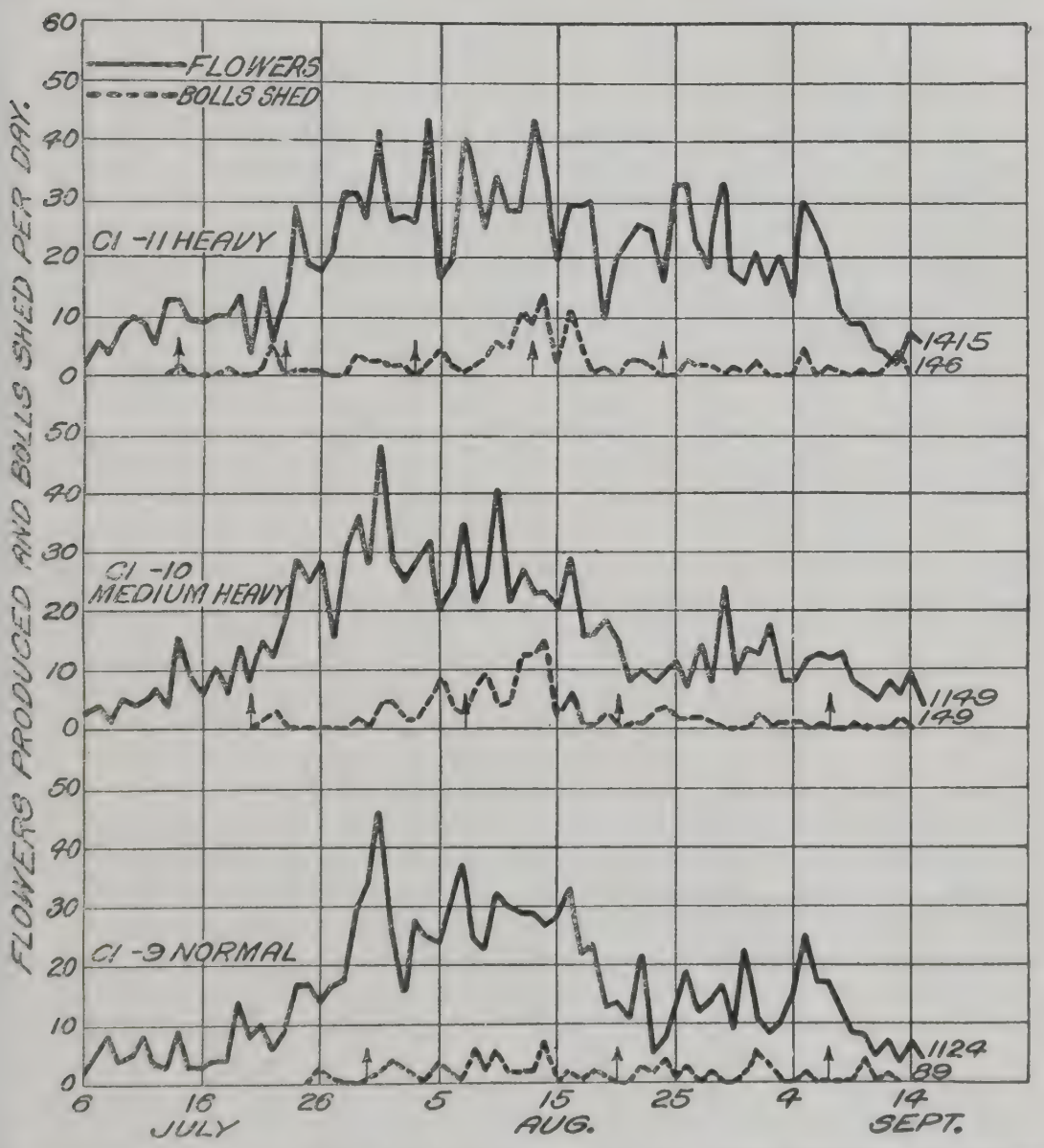

FIG. 4.—Numbers of flowers produced and bolls shed each day on 25-plant section of series II, compared with the irrigation dates. Note the lack of consistency of behavior at any regular period after irrigation. The arrows indicate irrigation dates.

tions shown in figure 3 give a general impression of periodicity, but there is no evidence of regularity. Also, the general consistency of flower production between borders would seem to indicate a short period between the determining factors, or stimuli, and the flowering dates; but with so little known regarding these features, it seems better to present the data without hazarding explanations.

TABLE VI.—*Numbers of flowers per day on the 25-plant section in each border*

Date.	Normal irrigation.				Medium-heavy irrigation.				Heavy irrigation.				Grand total.
	C1–3.	C1–9.	C1–12.	Total.	C1–4.	C1–10.	C1–13.	Total.	C1–5.	C1–11.	C1–14.	Total.	
July 6	2	2	2	6	3	3	1	7	3	2	0	5	18
7	8	5	1	14	8	4	4	16	3	6	4	13	43
8	4	8	1	13	4	1	1	6	2	4	7	13	32
9	1	4	0	5	4	5	2	11	3	8	2	13	29
10	8	5	4	17	3	4	9	16	4	10	3	17	50
11	10	8	7	25	11	5	6	22	6	9	9	24	71
12	7	4	2	13	6	7	7	20	0	5	0	5	38
13	12	3	4	19	19	4	12	35	[a] 10	[a] 13	[a] 5	28	82
14	10	9	10	29	13	16	8	37	11	13	13	37	103
15	11	3	8	22	9	9	11	29	10	9	3	22	73
16	11	3	7	21	7	6	4	17	6	9	6	21	59
17	17	4	5	26	10	10	14	34	9	10	4	23	83
18	9	4	9	22	13	6	3	22	12	10	4	26	70
19	11	14	10	35	15	14	12	41	19	14	9	42	118
20	14	8	6	28	[a] 10	[a] 8	[a] 8	26	9	4	12	25	79
21	20	10	7	37	18	15	17	50	14	15	10	39	126
22	5	6	8	19	6	12	8	26	8	6	14	28	73
23	16	9	21	46	17	19	18	54	[a] 20	[a] 14	[a] 9	43	143
24	19	17	10	46	22	29	20	71	18	28	15	61	178
25	28	17	24	69	21	25	17	63	27	19	20	66	198
26	23	14	19	56	24	28	13	65	20	18	19	57	178
27	18	17	10	45	25	16	20	61	18	21	25	64	170
28	20	18	30	68	17	31	25	73	21	31	31	83	224
29	31	29	27	87	40	36	31	107	20	31	21	72	266
30	30	[a] 34	[a] 30	94	24	28	30	82	22	27	23	72	248
31	33	46	41	120	39	48	34	121	37	42	34	113	354
Aug. 1	27	26	22	75	34	28	21	83	37	26	23	86	244
2	31	16	20	67	24	25	26	75	16	27	26	69	211
3	25	28	22	75	31	28	22	81	[a] 30	[a] 26	[a] 18	74	230
4	27	25	24	76	30	32	33	95	28	44	24	96	267
5	14	24	17	55	15	21	16	52	14	17	20	51	158
6	24	30	26	80	22	25	22	69	21	20	22	63	212
7	37	37	28	102	30	35	32	97	22	42	19	83	282
8	30	25	25	80	36	21	32	89	21	33	32	86	255
9	31	23	19	73	31	25	19	75	24	25	17	66	214
10	31	32	33	96	40	41	36	117	21	34	24	79	292
11	23	30	19	72	26	21	27	74	17	28	29	74	220
12	27	29	33	89	23	27	14	64	25	28	20	73	226
13	[a] 26	29	32	87	[a] 31	23	29	83	[a] 21	[a] 44	[a] 28	93	263
14	32	27	24	83	23	23	28	74	17	35	25	77	234
15	22	28	26	76	20	20	20	60	22	20	26	68	204
16	17	33	17	67	27	29	21	77	13	29	25	67	211
17	19	22	38	79	22	16	26	64	13	29	16	58	201
18	17	24	23	64	22	16	29	67	21	30	23	74	205
19	10	13	23	46	14	19	24	57	12	10	16	38	141
20	5	[a] 14	[a] 11	30	10	[a] 15	[a] 20	45	5	20	17	42	117
21	9	11	15	35	13	8	13	34	17	23	15	55	124
22	13	22	27	62	15	10	21	46	13	26	18	57	165
23	8	5	7	20	17	8	16	41	10	25	12	47	108
24	5	7	7	19	20	10	19	49	6	[a] 17	[a] 13	36	104
25	6	13	19	38	25	12	23	60	15	33	20	68	166
26	11	19	22	52	12	7	25	44	14	33	29	76	172
27	9	12	17	38	19	15	22	56	7	23	8	38	132
28	6	14	14	34	17	8	20	45	9	19	14	42	121
29	7	17	17	41	20	24	26	70	18	33	19	70	181
30	5	9	15	29	9	9	14	32	14	18	10	42	103
31	12	23	33	68	19	14	24	57	15	16	21	52	177
Sept. 1	8	11	14	33	8	13	14	35	14	22	14	50	118
2	9	8	20	37	8	18	16	42	10	16	14	40	119
3	6	10	18	34	16	8	26	50	[a] 13	21	11	45	129
4	2	15	13	30	9	8	10	27	8	14	11	33	90
5	5	25	19	49	10	12	17	39	8	30	19	57	145
6	10	17	16	43	15	13	25	53	10	26	10	46	142
7	8	[a] 17	[a] 9	34	9	[a] 12	[a] 10	31	11	21	8	40	105
8	7	12	8	27	10	13	12	35	10	12	4	26	88
9	4	8	12	24	8	8	13	29	10	9	3	22	75
10	4	8	5	17	7	7	8	22	4	9	0	13	52
11	1	4	4	9	5	5	1	11	5	5	1	11	31
12	4	7	7	18	8	8	11	27	6	4	1	11	56
13	4	3	3	10	5	6	4	15	4	2	4	10	35
14	6	7	2	15	4	10	7	21	7	7	2	16	52
15	3	4	3	10	2	4	6	12	4	6	2	12	34
Total	1,025	1,124	1,131	3,280	1,209	1,149	1,235	3,593	994	1,415	1,035	3,444	10,317
Average				1,093				1,197				1,148	

[a] Irrigation date.

Ewing [8] found in comparing the flowering curves of Trice cotton, from one plot on rich soil and another plot on poor soil, that—

> the same daily fluctuation in the flowering curve is noticeable in the two situations, and the most pronounced difference is in the height of the flowering curve.

This would agree with the results shown in figure 2, although instead of the soil being different the moisture supply was varied in our experiment, and as the moisture did not appreciably influence the rate of flowering the curves are not very different.

That the number of flowers per day was not noticeably affected by irrigation will be apparent from the diagram (fig. 4), which gives the daily flower production and the dates of irrigation for the three borders of series II. The behavior of the plants in this series is regarded as sufficiently representative of the other two series. There was no consistent behavior in either higher or lower rates of flowering at any regular interval after irrigation. This is in direct contrast to a common belief in the irrigated valleys of the Southwest that fewer flowers are produced for a few days after each irrigation, but no such relation is shown in the curves of figure 4.

It is recognized that the physical condition and size of the plants, fertility of the soil, and the amount of available moisture, undoubtedly are factors which limit the number of flowers produced; but from the data presented in figure 3 and figure 4, it seems that other influences not yet identified must determine whether the numbers of flowers on particular days shall be relatively high or low; and since it is possible to predict when a bud will flower one day and sometimes two days beforehand, it is evident that the determining influences are effective at least several days in advance.

SHEDDING OF YOUNG BOLLS

The shedding of young bolls began on July 14, but up to July 29 the rate was almost negligible, amounting to less than 1 shed boll per day for every 50 plants. A higher rate occurred during most of August, the highest number falling on August 12, but an average of the period when the maximum shedding occurred showed less than 1 shed boll per day for every 7 plants. During the latter part of August and the first two weeks in September the rate fell to less than 1 shed boll per day for 25 plants.

The daily shedding records for the 25-plant sections are given in Table VII, which gives the number of young bolls shed per day in each border, the total per day for all borders, the total per border for the season, and the percentage for each border.[9] The age of the bolls when the maximum shedding occurs is given in Table VIII, showing the number of days between the opening of the flowers and the shedding of the young bolls within a period of 30 days after flowering, with the number of bolls shed for each interval of days per border and the total for all borders.

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[8] EWING, E. C. A STUDY OF CERTAIN ENVIRONMENTAL FACTORS AND VARIETAL DIFFERENCES INFLUENCING THE FRUITING OF COTTON. Miss. Agr. Exp. Sta. Tech. Bul. 8, 93 p., 40 fig. 1918. Literature cited, p. 93.

[9] Diagrams of the shedding on series II, including borders C1–9, C1–10, and C1–11, from July 14 to September 15, derived from the data in Table VII, are shown in figure 3, where they are used for purposes of comparison with the flower diagrams and in relation to the dates of irrigation.

TABLE VII.—*Numbers of shed bolls per day and percentage of boll shedding for the season on the 25-plant section in each border*

Date.	Normal irrigation.				Medium-heavy irrigation.				Heavy irrigation.				Grand total.
	C1-3.	C1-9.	C1-12.	Total.	C1-4.	C1-10.	C1-13.	Total.	C1-5.	C1-11.	C1-14.	Total.	
July 14	1	.....	.....	1	.....	.....	.....	.....	.....	2	.....	2	3
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
16	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1	1
17	.....	.....	1	1	3	.....	2	5	.....	.....	.....	.....	6
18	.....	.....	.....	.....	1	.....	.....	1	1	1	.....	2	3
19	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
20	.....	.....	.....	.....	a 2	(a)	(a)	2	1	.....	.....	1	3
21	.....	.....	.....	.....	.....	2	1	3	.....	1	.....	1	4
22	2	.....	1	3	4	3	.....	7	3	6	.....	9	19
23	.....	.....	.....	.....	.....	.....	2	2	(a)	(a)	(a)	.....	2
24	1	.....	1	2	.....	.....	.....	.....	.....	1	1	2	4
25	.....	.....	.....	.....	1	.....	.....	1	.....	1	2	3	4
26	1	3	.....	4	1	.....	.....	1	.....	1	.....	1	6
27	2	1	.....	3	1	.....	.....	1	1	.....	.....	1	5
28	.....	.....	.....	.....	1	.....	.....	1	.....	.....	.....	.....	1
29	6	.....	2	8	4	2	1	7	.....	4	.....	4	19
30	2	a 1	(a)	3	1	.....	2	3	4	3	2	9	15
31	1	2	2	5	5	5	2	12	3	3	4	10	27
Aug. 1	4	4	4	12	7	5	2	14	6	2	4	12	38
2	5	3	1	9	4	2	4	10	4	2	4	10	29
3	1	2	.....	3	2	2	.....	4	a 1	(a)	a 3	4	11
4	1	1	2	4	1	5	1	7	2	2	1	5	16
5	6	4	3	13	12	9	7	28	22	5	13	40	81
6	4	2	8	14	5	4	1	10	5	2	3	10	34
7	1	1	1	3	7	3	2	12	4	1	5	10	25
8	4	6	5	15	4	7	6	17	6	2	4	12	44
9	1	2	2	5	2	10	4	16	1	3	8	12	33
10	8	6	6	20	2	4	6	12	6	6	4	16	48
11	4	2	3	9	2	5	3	10	2	5	2	9	28
12	7	2	11	20	19	13	8	40	13	11	8	32	92
13	a 6	2	3	11	a 2	13	1	16	8	9	.....	17	44
14	8	7	6	21	9	16	12	37	16	14	7	37	95
15	.....	.....	.....	0	.....	2	3	5	2	3	2	7	12
16	3	2	5	10	4	7	2	13	12	11	5	28	51
17	4	1	2	7	1	1	1	3	3	5	1	9	19
18	2	2	1	5	.....	1	2	3	.....	1	2	3	11
19	4	1	10	15	3	3	9	15	2	2	3	7	37
20	.....	(a)	(a)	0	2	(a)	(a)	2	1	.....	2	3	5
21	.....	.....	.....	0	3	1	.....	4	1	3	2	6	10
22	4	3	2	9	8	1	.....	9	3	3	2	8	26
23	3	2	1	6	2	3	.....	5	4	2	2	8	19
24	2	4	1	7	5	4	4	13	2	(a)	a 1	3	23
25	6	1	4	11	1	2	1	4	.....	.....	1	1	16
26	.....	3	8	11	1	2	6	9	.....	3	4	7	27
27	3	.....	.....	3	3	2	2	7	2	2	.....	4	14
28	2	2	.....	4	.....	1	1	2	1	2	1	4	10
29	.....	.....	.....	0	.....	.....	.....	0	.....	1	1	2	2
30	.....	.....	.....	0	1	.....	1	2	.....	2	.....	2	4
31	.....	2	1	3	1	.....	.....	1	.....	1	.....	1	5
Sept. 1	1	5	.....	6	.....	3	2	5	.....	3	3	6	17
2	.....	3	3	6	.....	.....	2	2	.....	.....	1	1	9
3	1	.....	.....	1	.....	1	.....	1	a 2	.....	.....	2	4
4	1	.....	1	2	5	1	.....	6	1	.....	.....	1	9
5	2	2	1	5	1	.....	.....	1	.....	5	1	6	12
6	1	.....	.....	1	.....	1	.....	1	.....	.....	.....	0	2
7	.....	(a)	(a)	0	.....	(a)	a 3	3	1	2	.....	3	6
8	.....	.....	.....	0	.....	.....	.....	0	1	1	.....	2	2
9	.....	.....	.....	0	.....	1	2	3	.....	.....	7	7	10
10	.....	4	1	5	2	.....	.....	2	1	1	3	5	12
11	.....	.....	1	1	.....	.....	1	1	.....	.....	.....	0	2
12	.....	1	1	2	.....	.....	2	2	.....	2	4	6	10
13	4	.....	2	6	3	2	4	9	2	4	2	8	23
14	1	.....	.....	1	.....	.....	.....	0	.....	.....	1	1	2
15	1	.....	.....	1	.....	.....	.....	0	.....	.....	0	0	1
Total shed	121	89	107	317	148	149	115	412	150	146	127	423	1,152
Total flowers	1,025	1,124	1,131	3,280	1,209	1,149	1,235	3,593	994	1,415	1,035	3,444	10,317
Percentage shed	11.8	7.9	9.4	9.7	12.2	12.9	9.3	11.4	15.0	10.3	12.5	12.6	11.2

[a] Irrigation date.

TABLE VIII.—*Number of bolls shed for each day after flowering*

Number of days after flowering.	Normal irrigation.				Medium-heavy irrigation.				Heavy irrigation.				Grand total.
	C1–3.	C1–9.	C1–12.	Total.	C1–4.	C1–10.	C1–13.	Total.	C1–5.	C1–11.	C1–14.	Total.	
1	.....	.....	.....	.....	.....	1	1	2	.....	.....	1	1	3
2	1	.....	.....	1	.....	1	.....	1	.....	1	1	2	4
3	1	1	1	3	.....	.....	.....	.....	1	3	3	7	10
4	5	2	2	9	5	5	4	14	8	5	6	19	42
5	8	2	7	17	7	14	4	25	7	4	4	15	57
6	4	9	10	23	11	20	6	37	11	16	11	38	98
7	12	8	11	31	13	8	15	36	14	15	18	47	114
8	15	7	10	32	21	18	13	52	12	14	12	38	122
9	10	10	18	38	11	15	6	32	14	15	11	40	110
10	9	6	10	25	15	9	10	34	15	14	10	39	98
11	7	8	6	21	9	13	6	28	8	10	13	31	80
12	7	1	6	14	10	8	7	25	12	9	6	27	66
13	3	4	4	11	13	9	8	30	15	6	.....	21	62
14	8	5	5	18	6	6	1	13	3	5	6	14	45
15	3	.....	2	5	3	4	7	14	3	3	7	13	32
16	4	.....	1	5	3	3	6	12	3	4	1	8	25
17	5	2	.....	7	2	2	2	6	3	6	4	13	26
18	3	4	3	10	2	.....	2	4	.....	3	.....	3	17
19	.....	4	1	5	3	2	3	8	3	1	.....	4	17
20	2	2	.....	4	1	.....	2	3	1	1	.....	2	9
21	3	.....	1	4	2	1	.....	3	1	1	1	3	10
22	1	.....	1	2	1	1	.....	2	2	2	.....	4	8
23	2	.....	1	3	.....	2	1	3	3	1	1	5	11
24	1	1	.....	2	.....	2	1	3	2	.....	2	4	9
25	1	.....	.....	1	.....	.....	1	1	2	2	1	5	7
26	.....	.....	.....	1	1	.....	.....	1	.....	.....	.....	.....	2
27	.....	1	1	2	.....	.....	3	3	1	.....	2	3	8
28	.....	.....	1	1	.....	1	1	2	2	1	.....	3	6
29	1	2	.....	3	.....	.....	.....	.....	.....	1	1	2	5
30	.....	1	1	2	1	.....	.....	1	1	.....	1	2	5

No significant differences appeared in either the amount of shedding per day or the total for the season as a result of the varied irrigation treatments. A comparison of the records in Table VII, shows that the number per day for one treatment is not consistently larger or smaller. Differences are shown both between the borders that represent the same treatment and between the series. The total number shed for the season and the percentage of shedding are less in the "normal" treated borders, but this may not be significant in view of the small number of plants included in the daily shedding record.

As can be seen by referring to Table VII, considerable variation occurred in the number of bolls shed from day to day throughout all the borders, but the amount of shedding on certain days was often noticeably consistent in all the borders. August 5, 12, and 14 are good examples of days when heavy rates of shedding occurred, while on August 3, 15, and 20 only a few bolls were shed. These variations may be analogous to the regular fluctuations found in the opening of the flowers, which has been discussed previously, but the limited amount of material is not sufficient to use as a reliable basis of comparison.

As with the data of flowering, efforts were made to correlate the fluctuations of shedding with temperatures and other factors, but such indications were not consistent enough to justify discussion. It seems probable that several factors must be taken into account in attempting to localize and determine the causes of shedding. That shedding may occur from 4 to 15 days after flowering or may not take place until several days after the abortion is induced is only one of the many difficulties to be met.

Shedding as a direct effect of irrigation was not indicated, even on the "heavy" borders, although it is generally believed that excess moisture may cause young bolls to drop. There was no generally higher or lower rate of shedding at any regular interval after a border was irrigated. This can be seen in figure 3, or by a comparison of the irrigation dates with the daily shedding record in Table VII. That the plants retained their normal fruitful behavior throughout the experiment instead of becoming overgrown is regarded as responsible for the lack of any excessive shedding. The highest shedding rate, 15 per cent, occurred on the "heavy" border C1–5, in which the plant growth was checked, apparently by too much water. But this rate of shedding was nearly equaled on the "medium-heavy" border C1–10, which showed no abnormal behavior.

The length of the period between the date of flowering and the date of shedding of young bolls was found to fluctuate, with no indication of being affected by irrigation as applied. It is shown in Table VIII that 82 per cent of the shedding occurred between 4 and 14 days after the flowers opened, with a maximum 8 days after flowering and 10.8 days as the average age of shed bolls. That so little shedding occurred until several days after flowering is of interest and would agree with results published by Lloyd [10] for Upland varieties, who found that "abscission is inhibited during anthesis," but there are differences between the Upland and Egyptian types with respect to the time of maximum shedding. Lloyd found under field conditions in Alabama that the period of greatest shedding of young bolls was from 4 to 8 days after flowering and that "the age at which bolls were shed in maximum numbers was 5 to 6 days," while his figures show that the average period of persistence was about 6 days. Thus there are definite indications that Upland bolls shed earlier in Alabama than Pima bolls in Arizona.

In 1918 Mr. C. J. King, of the Bureau of Plant Industry, found the mean period of persistence of shed bolls in Pima cotton at Phoenix, Ariz., to be 10.3 days after flowering,[11] which is in close agreement with the period of 10.8 days obtained in our experiment at Sacaton.

Under Sacaton conditions, the Egyptian cotton differs notably from the Upland varieties, which often shed a large proportion of their buds before flowering. This was especially noticeable in 1920, when two Upland varieties, Durango and Lone Star, were compared with Pima cotton. Until the latter part of the flowering season the great majority of the squares produced on the Upland plants were dropped, while the adjoining border of Pima cotton lost very few squares. As in the irrigation experiment, the shedding which took place in the Pima border was almost entirely of young bolls. Lloyd found in Alabama that about 50 per cent of the shedding in the Upland varieties consisted of squares.

Factors which caused shedding in the experiment were probably of a general nature, as the percentage of shedding was practically the same throughout the Pima cotton on the station that was receiving a normal treatment. Less shedding occurred than usual, which may be ascribed to the lack of heavy summer rains, the few rains being too slight to affect the amount of shedding. Lack of fertilization of the flowers caused

[10] LLOYD, Francis E. THE ABSCISSION OF FLOWER-BUDS AND FRUITS IN GOSSYPIUM, AND ITS RELATION TO ENVIRONMENTAL CHANGES. *In* Trans. Roy. Soc. Canada, ser. 3, v. 10, sect. 4, p. 55–61. 1916.
——— ENVIRONMENTAL CHANGES AND THEIR EFFECT UPON BOLL-SHEDDING IN COTTON. *In* Ann. N. Y. Acad. Sci., v. 29, p. 1–131, 26 fig. 1920. Literature, p. 129–131.

[11] KING, C. J. WATER-STRESS BEHAVIOR OF PIMA COTTON IN ARIZONA. U. S. Dept. Agr. Bul. 1018. p. 15. 1922.

little, if any, loss, as bees and wasps were numerous in the fields at all times and pollination was usually accomplished soon after a flower opened. Injurious insects were noticeably absent and none of the shed bolls were found to be damaged.

INDICATION OF OVERWATERING

It became apparent about August 18 that the plants in the "heavy" border in series I were in distress. Growth had practically stopped, and the rate of flowering showed a distinct decline. Many of the lower leaves were dying and dropping off, and the other leaves had an unnatural yellowish green color. This usually would be taken as a sign of urgent need of water, but since the soil was obviously wet, even on the surface, that explanation would not serve. This border was not irrigated with the other "heavy" borders on August 23. No indication of a similar condition could be found in the "heavy" borders in series II and III, although up to this time they had received the same irrigation treatment.

In view of the fact that no similar behavior was found on the adjacent borders or elsewhere on the station, it seems probable that excess moisture was causing the distress in border C1–5, because of some peculiarity of the soil in series I, which resulted in a lower water requirement. A comparison between the number of irrigations on the "normal" border in series I and the "normal" borders in series II and III shows that series I required only half as many irrigations. Thus the same effects as the irrigation of the "heavy" border in series I every 10 days might have been obtained in the "heavy" borders of series II and III by irrigation at 5-day intervals.

The affected border was irrigated on September 3, as the plants showed wilting in the forenoon although the ground still appeared moist. A crippled, or partially destroyed root system may possibly have been responsible for this wilting, which ceased after more water was given. On September 8 it was noted that the plants had improved in color and later that the color had continued to improve so that only a slight difference could be detected.

However, the setback this border had received was very noticeable and its recovery was too late to make good the handicap it had received, as can be seen from the plant growth data (fig. 2) and the picking record (Table IX).

YIELD

The record of seed cotton picked from each border is shown in Table IX, which also gives the number of plants per row and the number with vegetative branches. Four pickings were made, and the cotton from each row was weighed and recorded separately so as to allow an accurate evaluation of differences.

From Table IX it can be seen that the total seed cotton from the "normal" border in series I was 340.6 pounds, from the "medium-heavy" border 392.7 pounds, and from the "heavy" border 280.4 pounds. In series II the "normal" border yielded 267.5 pounds, the "medium-heavy" border 274.9 pounds, and the "heavy" border 265.9 pounds. From series III, the "normal" border totaled 254.3 pounds, the "medium-heavy" border 294.6 pounds, and the "heavy" border 302.2 pounds.

As shown in the foregoing data, the yields of seed cotton from the differently treated border varied to a considerable extent, but with no consistence in regard to treatment. The greater fertility of the soil is

responsible for the larger yields in series I. For reasons already explained the comparatively poor showing of the "heavy" border C1–5 was ascribed to overirrigation and resultant checking of the plants. The yields per border of series II are not significantly different. In series III the yields of the "heavy" and "medium-heavy" borders are almost the same, but considerably more than that of the "normal" border. Hence it is believed that the heterogeneity of the soil of the borders used for the experiment was responsible for the fluctuations rather than the varying irrigation treatments.

From the evidence obtained by a comparison of the yields of all the borders, and making allowances for the "heavy" border C1–5, it may be stated that the "normal" irrigation produced, on the average, about 30 pounds less seed cotton per border than either the "medium-heavy" or "heavy" irrigation, but that between the latter two no differences developed. This lower average yield of the "normal" borders indicates that this treatment may be underirrigation. In previous seasons one of the "normal" borders, C1–12, had yielded better than C1–13 and C1–14.

TABLE IX.—*Pounds of seed cotton per border and pounds per row for each picking*

Border.	Row	First picking, Sept. 17.	Second picking, Oct. 14.	Third picking, Nov. 15.	Fourth picking, Dec. 8.	Total.	Plants per row.	Plants with vegetative branches.
C1–3, "Normal"	1	7.3	16.8	14.4	6.1	44.6	240	96
	2	10.1	23.3	16.5	6.1	56.0	256	112
	3	9.5	18.2	15.1	6.3	49.1	241	109
	4	8.3	16.0	15.5	7.1	46.9	207	79
	5	7.3	17.3	14.8	8.4	47.8	283	111
	6	8.0	14.6	18.7	8.3	49.6	181	70
	7	6.9	17.2	15.2	7.3	46.6	245	98
Total	....	57.4	123.4	110.2	49.6	340.6	1,653	675
C1–4, "Medium-heavy"	1	5.0	14.4	18.2	7.3	44.9	287	100
	2	8.6	20.5	21.0	10.1	60.2	218	82
	3	9.1	18.8	21.0	11.8	60.7	258	91
	4	8.1	25.6	20.0	11.1	64.8	224	114
	5	7.4	18.0	19.0	10.9	55.3	296	97
	6	6.0	14.8	21.0	12.8	54.6	239	118
	7	6.4	16.2	20.0	9.6	52.2	253	88
Total	....	50.6	128.3	140.2	73.6	392.7	1,775	609
C1–5, "Heavy"	1	4.4	10.7	16.0	6.2	37.3	210	57
	2	5.0	14.7	18.0	8.0	45.7	292	90
	3	5.0	12.3	16.0	6.5	39.7	231	92
	4	5.1	12.1	15.0	5.9	38.1	250	59
	5	4.6	12.8	14.0	5.6	37.0	239	85
	6	9.1	16.9	17.0	6.7	49.7	239	78
	7	4.2	10.9	12.0	5.8	32.9	285	79
Total	....	37.4	90.3	108.0	44.7	280.4	1,740	540
C1–9, "Normal"	1	1.9	15.1	12.0	6.6	35.6	255	92
	2	3.2	17.1	15.0	6.8	42.1	250	74
	3	4.9	14.9	14.0	6.4	40.2	232	80
	4	3.5	15.9	14.0	8.1	41.5	317	72
	5	3.1	16.5	12.0	7.0	38.6	235	78
	6	4.0	15.0	9.0	4.7	32.7	264	58
	7	4.5	16.0	12.0	4.3	36.8	260	67
Total	....	25.1	110.5	88.0	43.9	267.5	1,819	527

TABLE IX.—*Pounds of seed cotton per border and pounds per row for each picking*—Con.

Border.	Row	First picking, Sept. 17.	Second picking, Oct. 14.	Third picking, Nov. 15.	Fourth picking, Dec. 8.	Total.	Plants per row.	Plants with vegetative branches.
C1-10, "Medium-heavy"	1	2.8	14.1	11.0	5.9	33.8	256	89
	2	2.9	14.6	12.0	7.2	36.7	271	90
	3	4.5	16.3	15.1	6.4	42.3	249	56
	4	4.4	15.2	10.0	7.0	36.6	227	73
	5	3.1	14.0	14.0	7.2	38.3	233	57
	6	4.2	17.9	16.0	9.2	47.3	254	49
	7	3.2	15.2	13.5	8.0	39.9	283	56
Total	....	25.1	107.3	91.6	50.9	274.9	1,773	470
C1-11, "Heavy"	1	3.3	13.1	15.5	5.3	37.2	232	68
	2	3.0	13.5	14.1	5.5	36.1	206	71
	3	3.2	13.8	17.2	4.9	39.1	231	74
	4	3.0	13.6	16.1	8.4	41.1	239	64
	5	2.9	13.6	16.8	6.1	39.4	264	56
	6	3.6	14.4	13.7	6.1	37.8	232	71
	7	2.4	11.6	14.7	6.5	35.2	258	59
Total	....	21.4	93.6	108.1	42.8	265.9	1,662	463
C1-12, "Normal"	1	4.3	15.0	12.0	4.9	36.2	272	48
	2	4.1	17.4	11.8	5.4	38.7	248	53
	3	3.0	14.4	11.5	4.7	33.6	245	52
	4	3.8	16.4	9.7	5.1	35.0	261	52
	5	3.5	14.3	10.0	4.1	31.9	225	39
	6	4.0	19.1	12.5	5.0	40.6	262	54
	7	4.4	15.9	10.9	7.1	38.3	241	61
Total	....	27.1	112.5	78.4	36.3	254.3	1,754	359
C1-13, "Medium-heavy"	1	3.4	16.8	14.5	7.2	41.9	295	44
	2	3.8	15.5	12.9	6.0	38.2	252	56
	3	4.6	17.9	15.5	6.0	44.0	250	63
	4	4.5	15.2	15.3	4.7	39.7	273	54
	5	6.0	18.1	14.2	5.9	44.2	265	61
	6	6.2	17.4	14.7	6.0	44.3	271	46
	7	4.2	15.4	16.8	5.9	42.3	270	55
Total	....	32.7	116.3	103.9	41.7	294.6	1,876	379
C1-14, "Heavy"	1	6.1	14.6	16.9	7.8	45.4	269	39
	2	4.3	16.0	15.5	8.3	44.1	247	31
	3	4.6	16.1	17.1	9.0	46.8	269	50
	4	4.6	13.9	16.8	6.5	41.8	228	74
	5	5.1	13.5	18.1	8.4	45.1	274	52
	6	5.1	13.9	17.7	8.9	45.6	262	54
	7	4.8	11.7	10.9	6.0	33.4	250	48
Total	....	34.6	99.7	113.0	54.9	302.2	1,799	348

A general agreement was found throughout pickings of the different treatments, the largest picking of the "normal" borders was always the second, that of the "heavy" borders the third, while from the "medium-heavy" borders the second and third pickings were about equal. It seems that the effect of more frequent applications of water tended to the later maturity of the crop.

CONCLUSIONS

Recognizing summer irrigation as a distinct problem, experiments were conducted at Sacaton, Ariz., to determine the effects of different frequencies of irrigation during the months of July and August on Pima Egyptian cotton that had reached a normal early fruiting stage.

Nine irrigated plots or "borders" were arranged in three series, affording three independent tests of contrasted irrigation treatmemts. Each series included a "normal" border irrigated according to the usual practice of applying water as indicated by the behavior of the plants, a "heavy" border receiving irrigations at frequent intervals, and a "medium-heavy" border receiving an intermediate irrigation treatment.

The behavior of the plants in each of the borders was observed and compared, and the data of the principal features, including growth, flowering, shedding of young bolls, and yields, were recorded through the season.

As a general result of the experiment it was determined that the different frequencies of irrigation, after the plants had reached a normal fruiting stage, did not cause any consistent significant difference in the growth of the plants, or in the yields.

No consistently different rates of flowering or of shedding of young bolls resulted from any of the different kinds of treatment.

A wide range in the daily flowering production occurred, there being days when all borders had large numbers of flowers and days when all borders had a few flowers. The rate of shedding of young bolls also varied from day to day, though on only a few days was there a consistent behavior throughout all the borders. No correlation between these fluctuations and factors which might be supposed to influence this phenomenon could be definitely established.

No consistent increase or decrease at a regular interval after an irrigation was found, either in the flowering or in the shedding of young bolls.

Shedding of young bolls was greatest from 4 to 14 days after flowering, the interval varying only slightly between borders. The shedding of flower buds, or "squares," was almost negligible throughout the experiment.

Plant development was checked and other symptoms of distress appeared in one of the three heavily irrigated borders, apparently from an excess of water.

The yields varied in the differently treated borders, but from heterogeneity of the soil rather than from the different frequencies of irrigation or in the loss of bolls by shedding.

The experiment indicates the importance of giving more attention to the spring treatment of cotton, so as to have the plants in a normal fruiting condition when summer irrigations begin. When this normal fruiting condition is attained, the summer irrigation problems are simplified, since the plants are not so easily forced into rank growth by the application of water in excess of the actual requirements.

PERMANENCE OF VARIETY IN THE POTATO[1]

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The practice of selection within the clone in order to maintain and improve the productivity and quality of potato varieties is of comparatively recent origin. In the early period of potato improvement, hybridization with subsequent selection of the most promising seedlings was the method employed. The practice of vegetative selection is founded for the most part on the success of numerous attempts to isolate high-yielding and low-yielding strains. Many investigators consider that the greatest benefit to be derived from selection is the maintenance of the optimum vigor of the variety, while other investigators believe that the results indicate the presence of inherited differences within the variety and have therefore considered selection to be of value in the improvement of varieties. Recent advances in the knowledge of certain diseases hitherto unrecognized have thrown considerable doubt upon the correctness of the latter interpretation. This study has for its primary object a determination of the effect upon the variety of selection as practiced by growers and a determination of the stability of regional differences, when the disease factor is eliminated.

REVIEW OF LITERATURE

The older theory suggested by Knight (*10*)[3] and Aitken (*1*) of varietal senility has given place to that of the deterioration of seed stock. Since selection has for one of its primary objects the prevention of this deterioration, a brief historical review of these theories will be of interest.

As early as 1787 William Marshall (*12*), writing from England, reported that potato varieties were transitory in each district. He noted that the declining varieties were characterized by curled tops. Cathcart (*3*) quotes Stephens' (*19*) "Book of the Farm" as authority for the statement that the condition of curl was first observed in 1764. Marshall (*12*) considered curl to be due to varietal senility, as it was noted that new varieties secured from seed were not subject to this condition. The following statement by Knight (*9*), in 1807, indicates the transitory nature of potato varieties at that time.

> Dr. Hunter [*8*] in his Georgical Essay, I think, has limited the duration of a variety in a state of perfection to about fourteen years; and probably taking varieties in the aggregate, and as the plant is generally cultivated, he is nearly accurate.

[1] Accepted for publication Feb. 15, 1922. Paper 298 of the Journal Series of the Minnesota Agricultural Experiment Station, published with the approval of the Director.

[2] The experiment was originated by A. W. Aamodt, who collected the original lots of potatoes, took descriptive data, and supervised the planting in the spring of 1917. The work was carried on under the supervision of Richard Wellington from the time of its inception until the spring of 1919. Thanks are due to O. I. Bergh, of the North Central Substation, Grand Rapids, Minn., Mark Thompson, of the Northeast Substation, Duluth, Minn., and Thomas McCall, of the Northwest Substation, Crookston, Minn., for their cooperation.

[3] Reference is made by number (italic) to "Literature cited," p. 959–961.

In a previous publication (*10*), Knight had put forward the theory of varietal senility, although he did not lay special emphasis on the potato. His hypothesis was that all plants propagated vegetatively have their period of maximum vigor when at middle age and then become—

subject within no very distant period to the debilities and diseases of old age.

Aitken (*1*) applied the hypothesis to potatoes in 1837, and it was accepted by many later writers. Ehrenburg (*6*), in 1904, after reviewing the work of previous investigators, concluded that the idea of varietal senility was untenable. East (*4*), in 1908, came to a similar conclusion and notes—

that the people who have considered this single question are unanimous in opposition to the hypothesis of varietal senility.

Ehrenberg (*6*) considered that the hypothesis of the deterioration of seed stocks being due to unfavorable environment has some basis for its support. East (*4*) regarded disease as the most important factor to be considered in connection with the degeneracy or running-out of seed stocks. Orton (*15*), in 1914, called attention to the so-called degenerative diseases of "mosaic," "curly-dwarf," and "leafroll" and to the probable part these diseases play in the running-out of seed stocks. The general distribution of these diseases and their harmful effect on the vigor of the plant, which has been brought out by later investigators—Stewart (*20*), Appel (*2*), Melhus (*13*), Murphy (*14, p. 33–82*), Güssow (*7*), Wortley (*30*), Shultz, Folsom, Hildebrandt and Hawkins (*17*), Quanjer (*16*), Shultz and Folsom (*17*), Krantz and Bisby (*11*)—has made it increasingly clear that the so-called running-out of seed is probably nothing more than a manifestation of these diseases.

Very little attention was paid to the possibility of improving varieties by means of asexual selection previous to the latter half of the nineteenth century. It has apparently never been considered worthy of serious attention by investigators in the British Isles. Sutton (*22*), in 1899, and Wilson (*29*), in 1907, in their discussion on the improvement of potatoes have expressed their belief that the seedling was a finished product and that further selection was of no value in its improvement. On the Continent and in the United States and Canada numerous investigators have attempted to ascertain its value. The meagerness of details in most of the earlier published reports on selection experiments makes them of little value in determining the place that selection occupies in the improvement of the potato. The most important papers on the value of selection have been reviewed by East (*4*), in 1908, and more recently by Stuart (*21*), in 1915. East (*4*), after reviewing the work of previous investigators, pointed out that the evidence was inconclusive and called attention to the influences which tended to obscure the results obtained by these investigators. Waid (*24*), in 1907, at the annual meeting of the American Breeders Association reported the results of studies on hill selection of seed potatoes. The results showed quite clearly that the progeny of low-yielding hills remained unproductive in comparison to the progeny of high-yielding hills. The favorable results reported in this work caused considerable emphasis to be placed on this method of improvement. The following year Webber (*25*) put forward a method of selection, known as the tuber-unit method, which has received considerable attention. East (*5*), in 1910, suggested that Waid's results might have been influenced by the size of the seed piece used and the fact that Waid might not have been dealing with a pure strain, as he

apparently used a commercial stock. East found no difference in yield between the progeny of high and low-yielding hills in stock which was obtained from a single tuber of the Rural New Yorker variety. He believes that great caution should be exercised in recommending asexual selection to commercial growers as a means of actual improvement of the crop, in view of the facts that of many investigations on the point no indisputable evidence of improvement has been reported and that even the questionable instances of positive results are extremely rare.

Stuart (*21*) reported in 1915 the results obtained in some rather extensive studies on the value of the tuber-unit and hill-selection methods as a means of potato improvement. Remarkable dissimilarities were found between individuals under both the tuber-unit and the hill-selection methods. Stuart did not attempt to explain the cause of the differences further than to point out that in certain instances—

> other causes than that of inherent unproductiveness must have operated to lower the yield,

and states—

> that the tuber unit method and hill selection method of improvement are chiefly valuable in pointing out the weak, unproductive, and diseased tuber.

Stewart (*20*) in his observations on some degenerate strains of potatoes states that—

> it is doubtful if any method of seed selection will prevent the "running-out" of seed potatoes under certain conditions.

In a recent article Whipple (*27*) has published the results of five years' work in pure-line selection. He calls attention to the persistency with which degenerate types (curly-dwarf) appear in pure lines and the consequent difficulty of interpreting yield data. Similar experiences have been recorded by Wellington (*26*) in a paper in which he points out the uselessness of hill selection under conditions where rapid deterioration or running-out is prevalent. The deterioration mentioned by Wellington was expressed by the production of the so-called "curly-dwarf" type of plants. This was later found by Krantz and Bisby (*11*) to be due to a disease very similar in its nature to mosaic and is probably nothing more than a severe expression of this disease.

A few experiments have been conducted to test the effect of asexual selection on characters other than yield. The results of a carefully conducted experiment for the purpose of determining the effect of selection for high and low nitrogen content was reported by East (*5*) in 1910. No difference in nitrogen content was found during three years of continuous selection. In conclusion East says—

> that neither the relative content of dry matter nor that of nitrogenous matters of the potato can be changed by the selection of fluctuations.

In attempting to isolate a blight-resistant strain of potatoes Stuart (*21*) obtained indifferent results. After selecting for three years to improve the form of the White McCormick variety, White (*28*) concluded that no improvement could be secured in this variety through selection. As previously mentioned, Stewart (*20*) and Wellington (*26*) were unsuccessful in trying to isolate a strain of potatoes resistant to factors causing degeneracy.

The evidence presented does not offer much encouragement to the plant breeder for the use of asexual selection for improving the potato. The opportunity that the mechanism of sexual reproduction offers for

the segregation and recombining of characters makes the method of hybridization with the subsequent selection of seedlings a more promising method for potato improvement. It has frequently been assumed that sufficient variation existed in all varieties to justify the grower in attempting improvement by asexual selection.

METHODS AND MATERIALS

In the fall of 1917 six lots of Early Ohio potatoes were secured from growers in different parts of Minnesota. Five of the lots were from growers who had practiced little or no selection. The sixth was from a grower who has practiced continuous mass selection of tubers for vigor and type in the same seed stock for approximately 20 years. In 1919 a seventh lot of Early Ohios was obtained from a grower who had practiced continuous mass selection of tubers for vigor and type in the same seed stock for 21 years. The growers differed in their ideas of what constituted the ideal type of the Early Ohio variety and had selected toward divergent types. Attention is called here to the fact that these two seed stocks were known to be separated for more than 20 years. This would seem to allow ample time for strains to develop within the seed stocks if there was a tendency for this condition to occur. The various lots represented distinct regional types found within the state. While it was recognized as a possibility that these seed stocks might not be asexual progeny of the same variety, it was still considered that a study of them would be of interest in determining the value of selection as practiced by the growers.

EFFECT OF SELECTION AND ENVIRONMENT ON THE POTATO PLANT

EFFECT ON PRODUCTIVITY

Results are presented in Table I showing the original source of the seed stock, the years in which the lots were tested, and their yield for each year at the places tested. The exact number of years the seed stock was grown at the place from which it was obtained is not definitely known, further than that lots 2 and 9 were grown at the places indicated for 20 or more years.

The 1917 test was carried on at University Farm, Duluth, Grand Rapids, and Crookston. The yields as presented in Table I, column III, are computed on the acre basis. The actual plot grown for each lot and locality consisted of a 4-rod row plot. The experimental error in such a test is large and the yield differences of the six lots grown are probably not significant as no lot was a consistent high or a consistent low yielder. In column IV are shown the results of the yields for 1918. These yields represented plots of three 4-rod rows computed to the acre basis. The three 4-rod rows represent seed stocks of each lot from three places—Duluth, Grand Rapids, and Crookston. The results are similar to those obtained in 1917 in that no lot gave a consistently high or low yield. In column V are shown the results obtained in 1919. The size of the plots at University Farm and Grand Rapids were the same as in 1918. At Duluth the yield given is the average yield computed to bushels per acre of two plots, each plot consisting of two 4-rod rows. As in 1917 and 1918, no lot gave a consistently high yield. Lot 7, however, gave a consistently low yield. The cause of this low yield is not clear. It could

hardly have been due to inherent difference in vigor from the other lots, since in 1917 and 1918 the yield of this lot was fully equal to that obtained from the other lots. In 1920 another lot designated as No. 9 was obtained and was grown in comparison with lots 2 and 7 at Grand Rapids. It gave an intermediate yield between these two lots.

The results of the 4-year test show no significant difference between the lots. They indicate that a significant difference probably did not exist. It is evident that the selected seed stocks 2 and 9 were not superior in vigor to the unselected seed stocks.

TABLE I.—*Productivity of Early Ohio Lots at University Farm, Duluth, Grand Rapids, and Crookston in 1917, 1918, 1920*

I, lot No.	II, source of seed, 1916.	III, yield, 1917.				IV, yield, 1918.				V, yield, 1919.			VI, yield, 1920.
		University Farm.	Duluth.	Grand Rapids.	Crookston.	University Farm.	Duluth.	Grand Rapids.	Crookston.	University Farm.	Duluth.	Grand Rapids.	Grand Rapids.
2	Anoka	205	203	326	44	255	158	286	.....	206	245	323	150
3	Grand Rapids	183	174	318	63	243	.....	.....	240	.....	.....	.....	.....
4	Faribault	216	174	311	80	272	185	.....	.....	.....	.....	.....	.....
6	Duluth	269	219	314	75	299	164	266	268	208	.....	300	.....
7	Glyndon	261	202	316	71	248	156	297	241	197	197	208	130
8	Hawley	259	179	265	56	258	.....	298	228	217	.....	249	.....
9	Hopkins [1]	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	147

[1] Obtained in 1919.

The effect of seasonal conditions on the yield of the progeny is of interest. In Table II is shown the yield of the same seed stocks in 1917, at Duluth, Grand Rapids, and Crookston, and their subsequent yield in 1918 at University Farm, Duluth, Grand Rapids, and Crookston. The yield is that of a 4-rod row plot computed to bushels per acre. Column III shows that all lots gave a very low yield at Crookston in 1917 in comparison to the yield obtained at Grand Rapids, and that a yield intermediate between that obtained at these two places was obtained at Duluth. Columns IV, V, VI, and VIII show the results obtained in 1918 in tests at University Farm, Duluth, Grand Rapids, and Crookston of the different portions of the lots grown at Duluth, Grand Rapids, and Crookston in 1917. The results given show that the excessive rainfall and an early frost which caused the low yield at Crookston in 1917 and made the tubers appear worthless for seed stock had no effect on their yielding ability in 1918.

EFFECT ON FORM OF TUBER

Of the various tuber characters that are usually collectively designated as type, that of form is one of the most important. Representative tubers of some of the original seed stocks are shown in Plate 1. Lot 2, obtained from Anoka, Minn., was oval in shape, being short and broad (Pl. 1, A). Continuous selection had been practiced by the grower toward this form of tuber, which is representative of the type obtained on the sandy loam soil of the region in which it was grown. Lot 3 was obtained from the North Central Experiment Station, Grand Rapids. The

tubers of this lot were relatively long and narrow. The Early Ohio potatoes produced at this station since that time, that is in 1917, 1918, 1919, and 1920, have been relatively short and wide. Lot 4 was obtained from Faribault, Minn. The tubers of this lot were elongated and were apparently somewhat variable in form (Pl. 1, B). A few tended toward an oval form. Lot 6 was obtained from the region about Duluth. The tubers of this lot were relatively short, very broad, and considerably flattened (Pl. 1, D). Lots 7 and 8 were obtained from growers in the Red River Valley. The tubers of these lots were characteristic of the more elongated cylindrical form of the Early Ohio of this region (Pl. 1, C).

TABLE II.—*Showing that poor growth conditions at Crookston in 1917 as compared to Duluth and Grand Rapids did not affect the yield of Crookston stock in 1918*

I, lot No.	II, source of seed, 1916.	III, yield, 1917.			IV, yield at University Farm, 1918 of seed grown in 1917 at—			V, yield at Duluth in 1918 of seed grown in 1917 at—			VI, yield at Grand Rapids in 1918 of seed grown in 1917 at—			VII, yield at Crookston in 1918 of seed grown in 1917 at—		
		Duluth.	Grand Rapids.	Crookston.	Duluth.	Grand Rapids.	Crookston.	Duluth.	Grand Rapids.	Crookston.	Duluth.	Grand Rapids.	Crookston.	Duluth.	Grand Rapids.	Crookston.
2	Anoka	203	326	44	293	271	202	193	138	143	290	299	269	.....	.....	.....
3	Grand Rapids	174	318	63	242	224	263	.....	.....	.....	.....	.....	.....	249	220	249
4	Faribault	174	311	80	288	224	315	220	110	146	.....	.....	.....	.....	.....	.....
6	Duluth	219	314	75	297	293	308	165	158	169	246	247	311	301	249	255
7	Glyndon	202	316	71	346	227	271	152	178	139	256	266	359	273	155	299
8	Hawley	179	265	56	264	218	288	.....	.....	.....	314	255	256	258	191	235

A part of each of these lots were grown at University Farm, Duluth, Grand Rapids, and Crookston in 1917. The form of the tubers produced at each of these places was similar in all the lots. It was evident that regardless of the form of the tubers in the original lots the growth conditions at each place produced a uniform distinctive form of tuber. The 1917 crop was carefully studied for possible differences in form between the lots grown at any one place. It was thought that slight differences in form were observable between some of the lots grown at Duluth. These differences in form were not found to remain consistently in 1918, although a similar amount of variation between the lots was observable.

In 1919 a study was made of the ratio of width of tubers to length in order to determine its value as an index to tuber form. Correlation studies for the ratio of width to length of tubers are presented in Tables IV to VII. The coefficients are here summarized.

TABLE III.—*Relation between width and length of tubers in the Early Ohio variety in 1919*

Place grown.	Coefficient of correlation.	Number of individuals.
Grand Rapids	0.718±0.014	571
Duluth	.847± .025	316
University Farm, clay loam	.647± .032	148
University Farm, sandy loam	.601± .034	161

The data presented in these tables show that there is a decided correlation between the width and the length of tuber and that this correlation

is similar for both small and large tubers of the same lots. The ratio of width to length of tuber would therefore appear to be a very good index of form in the Early Ohio variety. This is more clearly shown in Table VIII, which shows the influence of growth conditions on the form of tubers.

TABLE IV.—*Correlation between width and length of tuber in the Early Ohio variety, grown at the North Central Experiment Station, Grand Rapids, in 1919*

[Coefficient of correlation=0.718±0.014]

	Length of tubers in millimeters.																						Total.
	46	50	54	58	62	66	70	74	78	82	86	90	94	98	102	106	110	114	118	122	126	130	
40	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
42	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
44	...	...	2	...	...	1	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	5
46	...	1	1	10	2	1	1	2	...	1	...	...	...	...	...	...	...	...	...	...	...	...	19
48	...	...	1	9	7	5	2	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	26
50	...	1	4	7	10	9	8	3	...	1	1	...	...	...	...	...	...	...	...	...	...	...	44
52	...	...	3	7	15	12	14	2	5	2	1	...	...	...	...	...	...	...	...	...	...	...	61
54	...	...	1	2	9	17	14	7	6	9	2	1	...	...	...	...	...	...	...	...	...	...	68
56	...	1	...	3	4	7	12	9	9	...	3	2	1	1	1	...	...	...	...	...	...	...	53
58	...	...	...	1	...	3	14	14	15	11	6	5	4	...	2	...	...	...	...	...	...	...	75
60	...	1	...	1	...	5	10	7	12	10	8	8	2	2	1	...	...	...	...	...	...	...	67
62	...	...	...	1	1	2	1	7	4	7	6	4	3	3	3	...	1	2	...	...	...	...	45
64	...	...	...	...	...	...	3	...	5	5	4	2	1	1	4	1	1	...	...	...	...	...	28
66	...	...	...	...	...	2	...	2	...	1	2	1	6	2	1	1	...	...	...	...	...	...	18
68	...	...	...	...	...	...	1	1	...	1	4	2	3	1	2	...	3	1	...	...	...	...	19
70	...	...	...	...	...	...	...	...	...	2	1	3	2	2	2	...	...	2	1	1	...	...	16
72	...	...	...	...	...	...	...	...	...	...	...	2	1	2	1	1	0	...	1	0	...	1	9
74	...	...	...	...	...	...	1	...	...	1	...	1	...	...	2	...	...	...	...	...	...	...	5
76	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	...	1	...	1	...	...	...	4
78	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	1
80	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	...	...	...	...	...	2
82	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1	...	...	...	3
84	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
Total	...	6	12	42	48	64	82	55	58	51	38	33	25	15	20	3	8	6	4	1	...	1	571

TABLE V.—*Correlation between width and length of tuber in the Early Ohio variety, grown at Northeast Experiment Station, Duluth, in 1919*

[Coefficient of correlation=0.847±0.025]

Width of tubers in millimeters.	Length of tubers in millimeters.																						Total.
	46	50	54	58	62	66	70	74	78	82	86	90	94	98	102	106	110	114	118	122	126	130	
44	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
46	...	1	2	...	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	6
48	1	5	5	6	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	18
50	...	4	7	4	2	1	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	20
52	2	1	1	9	6	2	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	24
54	...	...	6	5	14	4	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	32
56	...	...	2	7	1	13	7	2	...	...	...	1	...	...	...	...	...	...	...	...	...	...	33
58	...	...	...	5	8	7	6	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	30
60	...	...	...	1	5	7	6	4	2	...	...	1	...	...	...	...	...	...	...	...	...	...	26
62	...	...	...	2	2	6	3	6	2	2	2	...	...	...	...	...	...	...	...	...	...	...	26
64	...	...	...	...	2	5	3	6	2	2	2	...	...	...	...	...	...	...	...	...	...	...	22
66	...	...	...	...	2	2	1	7	1	3	2	...	...	...	...	...	...	...	...	...	...	...	18
68	...	...	...	1	...	1	1	4	3	2	2	1	1	1	...	...	...	...	...	...	...	...	17
70	...	...	...	...	...	1	1	...	1	4	...	...	...	...	...	...	...	...	...	...	...	...	7
72	...	...	...	...	...	...	1	...	...	...	4	1	...	1	2	...	...	...	...	...	...	...	9
74	...	...	...	...	...	...	...	...	1	3	1	3	1	...	...	1	...	...	...	...	...	...	10
76	...	...	...	...	...	...	...	...	...	...	1	1	1	...	...	...	...	...	...	...	...	...	3
78	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	...	...	...	...	...	2
80	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
82	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	...	...	2
84	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
86	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	...	2
88	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	1
90	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
92	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1
Total	3	12	23	40	45	50	36	34	13	16	14	9	3	4	3	2	2	1	...	...	...	...	316

TABLE VI.—*Correlation between width and length of tuber in the early Ohio variety, grown on clay loam soil at Central Experiment Station, University Farm, St. Paul, in 1919*

[Coefficient of correlation 0.647±0.032]

Width of tubers in millimeters.	Length of tubers in millimeters.																						Total.
	46	50	54	58	62	66	70	74	78	82	86	90	94	98	102	106	110	114	118	122	126	130	134	
42																								
44				1	1		1																	3
46				1	1	1	2	1	2															8
48					2	4	4	1	1	1														13
50			1	1	1	1	2	1		3														10
52				1		2	1	2	2		3	3	1											15
54				1	2	2			5	3	1	1	1											16
56				1				4		4	1	1	1											12
58			1					2	1	5	3	4		1	2	1								20
60										3	1	3			2									9
62					1				2	1	1	3	1			1	1							11
64									2	1	1	2	2			1	1	1						11
66							1					1		2	2			1						7
68											2			1										3
70											1	1		1										3
72										1														1
74														2										2
76														1			1							2
78													1											1
80																								
82																								
84													1											1
Total			2	6	8	10	11	11	15	22	14	19	8	8	6	3	3	2						148

TABLE VII.—*Correlation between width and length of tuber in the early Ohio variety, grown on sandy loam soil at Central Experiment Station, University Farm, St. Paul, in 1919*

[Coefficient of correlation 0.601±0.034]

Width of tubers in millimeters.	Length of tubers in millimeters.																						Total.
	46	50	54	58	62	66	70	74	78	82	86	90	94	98	102	106	110	114	118	122	126	130	134	
42	1					1		1																3
44						5	1	2			1						1							9
46					1		5	2	1	1	1	1												12
48					1	1	1	5	2	3	1		1											15
50						1	3	3	1	3	5		1											17
52							1	1	3	2	4	2	3	2										18
54				2				1		1	2	1	2	1										10
56								1	1	1	3	1	1	2	1	2	1							14
58							2	1		1	1	1	3	3	1	3		1	1					18
60									1		1	2			2	1								7
62												1		3	2	1	2	1						10
64										1	1					2		1						5
66												1	1			1		2			2		1	8
68																1		2						3
70													1					1	2		1			5
72																	1		1					2
74																		2						2
76														1										1
78																								
80																							1	1
82																								
84																						1		1
Total	1			2	2	8	13	17	9	13	20	10	13	12	6	11	4	10	4		3	1	2	101

TABLE VIII.—*Effect of growth conditions on the form of Early Ohio Tubers*

Lot No.	Place grown.	Year.	Form index $\left(\frac{\text{length}}{\text{width}}\right)$ classes.							Total number of tubers.	Mean index.
			0.90	1.10	1.30	1.50	1.70	1.90	2.10		
2	Duluth	1919	14	110	38	2	1	....	....	165	1.14±0.01
2	University Farm sand loam	1919	....	1	3	17	25	13	1	60	1.65± .02
2	University Farm, clay loam	1919	....	13	17	30	44	10	1	105	1.59± .01
2	Grand Rapids	1919	....	22	67	39	12	1	....	141	1.36± .01
7	Grand Rapids	1919	....	15	67	30	11	1	....	124	1.36± .01
2	Grand Rapids	1920	1	28	38	4	....	....	....	71	1.23± .01
7	Grand Rapids	1920	1	14	30	1	....	....	....	45	1.23± .01

Table VIII shows the number of the lot, the place grown, the year, the frequency distribution according to the index of width to length, and the mean index for each lot. Early Ohio tubers grown at Duluth in 1919 were relatively short, very broad, and somewhat flattened, having a mean ratio of width to length of 1 to 1.14. An example of this form of tuber is shown in Plate 1, D, which shows the original form of lot 6 as obtained from Duluth in 1916. At University Farm, in 1919, on sandy loam soil the tubers were elongated, cylindrical in form, with a mean ratio of width to length of 1 to 1.65. On clay soil at University Farm the tubers were relatively thicker, broader, and shorter than those produced on the sand, having a ratio of width to length of 1 to 1.44. At Grand Rapids the tubers tended to be somewhat similar in form to those grown at Duluth but had less breadth and were slightly more elongated, having a mean ratio of width to length of 1 to 1.35 in 1919 and 1 to 1.23 in 1920. The difference in form obtained in 1919 and 1920 shows the effect of seasonal conditions on the form of tuber.

The results presented show that the mean index of the ratio of width to length of tubers can be used to detect changes in the form of tubers of the Early Ohio variety.

This method was used in 1919 and 1920 in ascertaining whether any difference in form of tubers existed between the lots of Early Ohio which were being tested. The results are presented in Table IX, which gives the number of the lots, the place and year grown, the frequency distribution according to their index number of width to length and the mean index number of each lot.

TABLE IX.—*Variation in form between different lots of Early Ohio potatoes in 1919 and 1920*

Lot No.	Place grown.	Year.	Form index $\left(\frac{\text{length}}{\text{width}}\right)$ classes.						Total number of tubers.[1]	Mean index.
			0.90	1.10	1.30	1.50	1.70	1.90		
2	Grand Rapids	1919	....	22	67	39	12	1	141	1.36±.01
6	.....do	1919	2	58	103	59	7	....	229	1.31±.01
7	.....do	1919	....	15	67	30	11	1	124	1.36±.01
8	.....do	1919	1	28	69	37	4	1	140	1.33±.01
2	Duluth	1919	14	110	38	2	1	....	165	1.14±.01
7	.....do	1919	12	83	83	9	....	1	188	1.20±.01
9	Hopkins	1919	....	1	2	15	10	2	30	1.57±.02
2	Grand Rapids[1]	1920	1	28	38	4	....	....	71	1.23±.01
7	.....do[1]	1920	1	14	30	1	....	....	45	1.23±.01
9	.....do	1920	3	32	40	9	....	....	84	1.23±.01

[1] From seed stock grown at Duluth in 1919.

The mean of lots 2, 6, 7, and 8 grown at Grand Rapids in 1919 ranges from 1.31±0.01 to 1.36±0.01. The mean of lots 2 and 7 grown at Duluth in 1919 was 1.14±0.01 to 1.20±0.01, respectively. The difference between the lots at Grand Rapids is 3.5 times the probable error and between the two lots grown at Duluth 4.2 times the probable error. This would appear to be significant. That the difference is probably due to other causes than hereditary is indicated by the fact that lots 2 and 7, between which the greatest difference occurred when grown at Duluth, gave the same mean index, 1.36±0.01 at Grand Rapids, and when the portions of the lots grown at Duluth were tested out in 1920 at Grand Rapids they gave an identical mean index, 1.23±0.01. In 1920, lot 9 was obtained from a grower who had selected for a somewhat more elongated form of Early Ohio. The lot as obtained in the fall of 1919 gave a mean of 1.57±0.02. It was grown at Grand Rapids in 1920 in comparison with lots 2 and 7. The mean index obtained for the three lots was 1.23±0.01. The results of the four years' study on the form of tubers show that the six lots gave an identical reaction, as regards the form of the tuber, when grown under similar growth conditions. The mass selection practiced by the growers in lots 2 and 9 for 21 years had no demonstrable effect on the inheritable form of Early Ohio tubers. It was further found that the form of tubers was very distinctly influenced by the environmental conditions surrounding the development of the tubers. This influence was not found to be carried over in any detectable amount to the progeny. As has been pointed out, the environmental conditions which resulted in good or poor yields did not measureably affect the yield of the progeny. It is therefore evident that no correlation was found between the form of tubers and their yield.

EFFECT OF ENVIRONMENT ON OTHER CHARACTERS

The formation of knobs is an undesirable character. This fact has been so obvious to potato breeders that the expression of this character by seedlings resulted in their rejection, so that our commercial varieties do not form knobs when growth conditions are uniformly favorable

throughout the season. When growth conditions are such as to cause a second growth of the plant, the formation of knobs frequently occurs on the tubers of some varieties. In the original lots of Early Ohio secured in the spring of 1917 all were comparatively free of knobs except lot 8. Traces of knobs had formed on all but lot 2. The progeny of these lots when examined in the fall of 1917 were found to be similar for this character in all lots grown at the same place. Knobs were present to a greater or lesser degree on the tubers of the lots grown at Grand Rapids, University Farm, and Crookston. Those grown at Duluth were free of knobs. In 1918 all lots at University Farm produced knobs, while at Duluth, Grand Rapids, and Crookston potatoes were free of knobs. The six lots appeared to give a similar reaction in regard to the formation of knobs under similar environmental conditions.

Another undesirable character is the formation of fissures or crevices on the surface of the tubers. The tubers of the original lots of Early Ohio were free of these fissures. Tubers containing fissures were frequently found in all lots grown at University Farm in 1917 and 1918. In 1917 they were occasionally found in tubers of lots grown at Crookston. At no time were fissures found in tubers of lots grown at Duluth and Grand Rapids.

The depth of eyes and prominence of eyebrows were found to be similar for the progeny of all lots grown at the same place. Distinct differences in the expression of these characters were produced at the different places, but this difference in expression had no influence on the progeny, as the characters were again expressed according to the environmental conditions under which the tubers developed.

The surface of the skin of the Early Ohio tuber is covered with small corky dots or lenticels. De Vries (*23*) has shown that the lenticels on potato tubers are due to the growth of loose cells underneath the stomates, which push up through the stomates and rupture them. He pointed out that a prominent development of them could be secured by having the tuber under moist conditions. These dots either do not occur at all or are relatively inconspicuous on varieties belonging to the Rose group. The original lots differed in the number and prominence of these lenticels on the tubers. The lenticels were very prominent on the progeny of all lots grown at University Farm in 1917, slightly less prominent on those grown at Crookston, slightly prominent on those grown at Grand Rapids, and very inconspicuous on those grown at Duluth. The result was similar in 1918.

In the original seed stocks the tuber color was distinctly different for each lot. The difference did not reappear in the progeny of the lots when grown under similar conditions. The growth conditions at each place produced a characteristic color of tubers. At times there appeared to be a difference between lots grown at the same place, but no lot was consistently different from the others in this respect. There was always considerable variation between individual tubers of the same lot. In general the larger tubers appeared less colored than the smaller ones. The color in the tuber of this variety is in the tissue directly beneath the cells forming the outer skin. De Vries (*23*) called attention to the fact that in the last growth of the skin during the ripening period the skin, which is previously clear, transparent, and smooth becomes thick and opaque. In the lots of Early Ohio under observation the difference in the amount of color present may have been due

to the degree to which it was masked. The vines, especially at Duluth, Grand Rapids, and Crookston, were usually killed by frost before the ripening period was completed, so that the stage of maturity probably influenced the color appearance of the tuber. The type of soil at a particular place also had a noticeable effect on the skin, as, for instance, tubers grown in sandy loam soil at Grand Rapids possessed a flakiness of skin whereas those grown at Duluth on clay soil containing a plentiful supply of humus were extremely smooth. A similar smoothness was obtained on tubers of the same stock when grown on peat at the Coon Creek Peat Experimental Farm at Anoka. These and other factors which influence the texture and quality of the skin were probably the cause of some of the difference in the color of the tubers.

CONCLUSION

The lots of Early Ohio potatoes were, as far as could be ascertained, identical in their characteristics. The number of lots under observation was relatively small. It must, however, be considered that each lot was chosen because it offered a possibility of being a distinct strain. Furthermore, two of the seed stocks were known to have been separated from each other and from the other seed stocks studied for at least 20 years. As the Early Ohio originated approximately 50 years ago, 20 years would seem to be sufficient time to allow strains to develop within the seed stocks of a variety, if such a tendency existed. The selection practiced by the growers with divergent types in view should further have tended to bring out any differences that might have developed. Also the difference in environment was sufficiently great to bring out distinct differences in the expression of characters studied. If the environment had any tendency to influence the expression of the character in the progeny, its influence should have been apparent at the end of this period. The evidence presented indicates that the potato variety is relatively stable under vegetative propagation. While mutations are known to occur, they are apparently not sufficiently numerous to offer reasonable hope for the further improvement of varieties by asexual selection. This does not mean that the methods of asexual selection are of no value in the improvement of commercial seed stocks. The use of the individual-hill and tuber-unit methods of asexual selection in the seed plot aid in the elimination of varietal mixtures, and by separating the progeny of diseased and healthy tubers in the seed plot are of aid in the roguing out of diseased plants.

SUMMARY OF EXPERIMENTAL RESULTS

(1) Seven lots of Early Ohio potatoes representing difference in amount of selection practiced by growers and distinct regional types were obtained from growers at Anoka, Duluth, Grand Rapids, Glyndon, Hawley, Faribault, and Hopkins, Minn. Observations were made on the behavior of the lots in 1917, when grown in comparison with each other at University Farm, Duluth, Grand Rapids, and Crookston. In 1918 the lots were under observation at two or more places. On some lots observations were continued in 1919 and 1920.

(2) In 1917 there was a difference of 20 bushels per acre between the average yield at the four places of the highest yielding and lowest-yielding lot. No lot consistently gave either a high or low yield at more

than two places. Lot 6, the highest-yielding lot in 1917, gave the highest yield in 1918, but in 1919 it was outyielded by lot 2, the lowest-yielding lot in 1917. Lot 9, obtained in 1920, gave a yield almost identical with that of lot 2. Selection practiced by the growers on lots 2 and 9 had apparently no effect on increasing their productivity.

(3) The ratio of width to length of tuber was found to be a fairly good index by which differences in form could be detected in the Early Ohio variety. It furnished a mathematical expression by which the form of tubers grown under different conditions and during different seasons could be compared.

(4) The original lots differed in form. The most distinct differences were short, oval tubers, short, broad, flat tubers, elongated, cylindrical tubers, and distinct modifications of these three forms (see Pl. 1, A, B, D, C). When grown at University Farm, Duluth, Grand Rapids, and Crookston a distinct form which was the same for all lots was obtained at each place. At Grand Rapids it was shown that seasonal conditions affected the form. At University Farm, sandy loam and clay loam soil types produced distinct differences of form. No correlation between form and yield was observed.

(5) Knobs, fissures, prominence of lenticels, depth of eyes, prominence of eyebrows, and color of tubers were characters that were found to be unexpressed, expressed, or modified in their expression according to the environment under which the tubers developed. The expression of these characters on the tuber was not influenced by selection or by the environmental conditions surrounding the development of the seed stocks.

(6) Evidence presented shows that potato varieties do not run into definite strains, that they are relatively stable under vegetative propagation, and that the method of asexual selection does not offer reasonable hope for their further improvement.

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PLATE 1

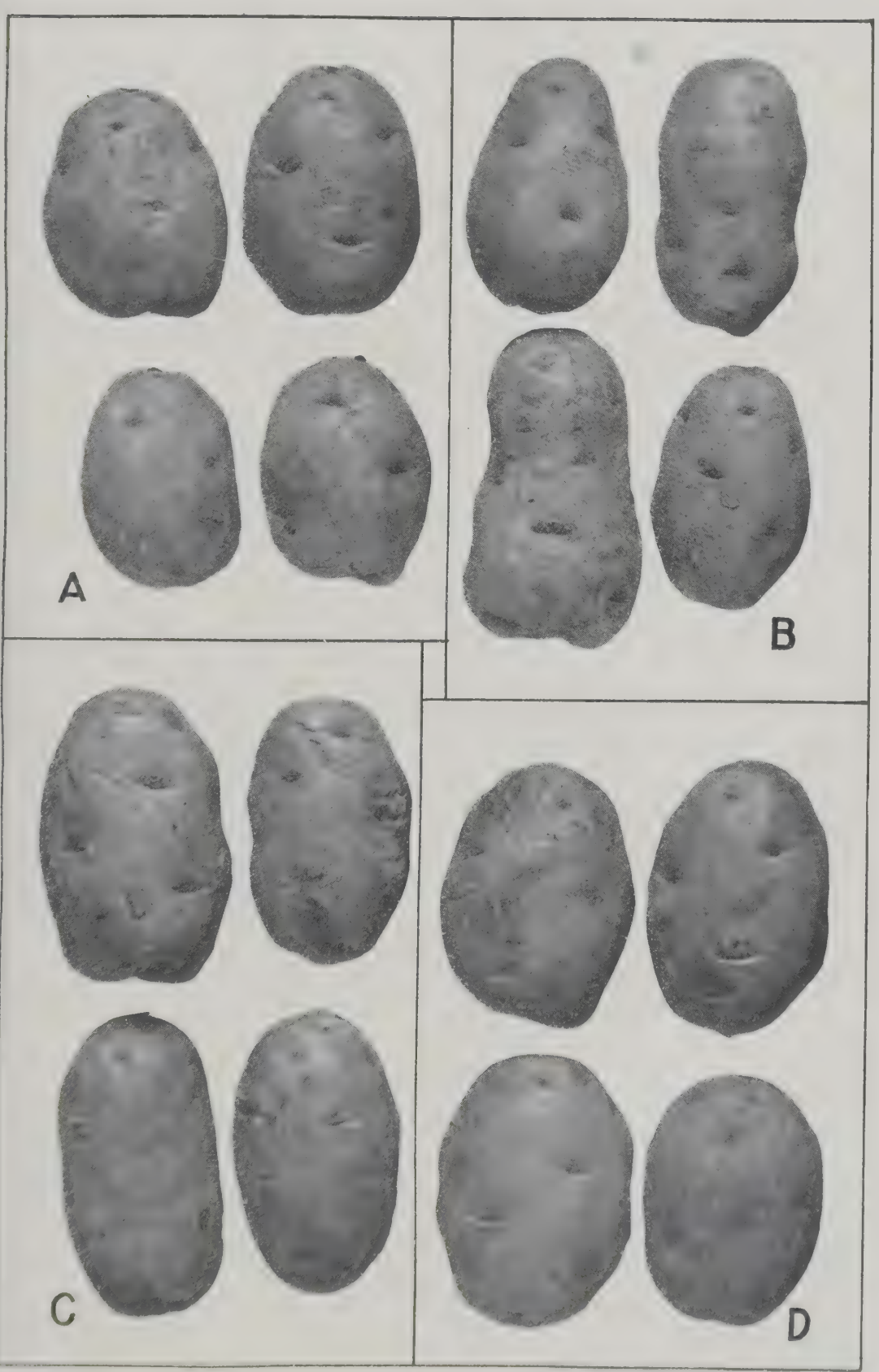
Potato tubers showing some of the variations existing between the original lots of Early Ohio. The progeny of these lots were found to be similar when grown under similar growth conditions.

A.—Short, oval, cylindrical tubers of lot 2 as obtained from Anoka in 1916.

B.—Somewhat elongated, irregular tubers of lot 4 as obtained from Faribault in 1916.

C.—Elongated, somewhat flattened tubers of lot 7 as obtained from the Red River Valley in 1916.

D.—Short, broad, flattened tubers of lot 6 as obtained from Duluth in 1916.


A
B
C
D

ANATOMICAL STUDIES ON POTATO-WART[1]

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Considerable progress has been made in recent years in the study of the causal organism of potato-wart, but our knowledge of the anatomical structure of the tumor is still limited to brief accounts found in earlier publications. A study of the histology of the tumor, though primarily of interest to the anatomist, will indirectly advance other phases of research in connection with the wart disease.

The wart appears as a proliferation of meristematic regions of stem, stolon, and tuber. An examination of a partly mature plant, grown under conditions favorable for infection, will give a picture such as is presented in Plate 1. Warts are seen to have developed abundantly on all underground parts except the roots. The tumors vary in size from minute undifferentiated protuberances to intricate branch systems. The largest warts have developed on the underground stalk and terminally on stolons, while the smallest appear on tubers, usually as the result of belated infections.

The typical wart is nearly spherical in form and gives the impression of a solid structure with modified peripheral parts. These external modifications, however, are the visible external expression of deeply seated changes so intricate (Pl. 2, E) that only the aid of the microscope will reveal their component parts. The wart, indeed, consists fundamentally of depressed, antler-shaped branches or metamorphosed leaves, partly grown together and branching profusely near the periphery. Since the planes of branching change constantly, symmetrical expansion of all the component branches in a centripetal manner is impossible; as a result the parts are greatly malformed and bear but little resemblance to any normal organ of the plant.

When tuber infection is belated and conditions are generally unfavorable for the development of the parasite the growth of the wart comprises only a superficial modification of the parts attacked (Pl. 3, C, D). While this type of wart may be found on any diseased plant it constitutes the only type which is developed on semiresistant varieties.

Wart formation on aerial organs is uncommon, but occasionally stem buds close to the surface of the ground become infected. Since in these buds leaf differentiation has usually gone past the embryonic stage, even the stimulating effect of the parasite can not altogether change the subsequent development of these organs. The result is a malformed leaf with a lamina little expanded but thickened (Pl. 2, B).[2]

[1] Accepted for publication May 17, 1922.

[2] Mr. Freeman Weiss reports having found a small, roughly spherical wart originating from the midrib of the lower surface of a leaf 12 to 14 inches above the ground; it contained both resisting spores and sori in various stages of development.

The color of the wart below ground is that of the stolon and young tuber, commonly an ivory white. In varieties which produce highly colored tubers, like the Australian Blue, the color of the wart will also be that of the tuber. When developing above ground, the parts exposed to the sun develop chlorophyll and aid in assimilation. Toward the end of the growing season, or earlier, the warts show signs of decay. The color changes from white to a dark brown and finally to a dirty black. At the time the potatoes are ordinarily dug, most large warts have disintegrated, leaving only unsightly vestiges on the infected tubers. Small warts, according to Johnson(*6*),[3] may enter a dormant state and form the source for new infections the following season.

ANATOMY

A cross section of a young wart or of the peripheral part of an older one shows a centrally located vascular strand (Pl. 2, D) and a broad band of cortical tissue whose peripheral cells harbor the resting spores of the parasite (Pl. 4, B). The epidermis, where still intact, is composed of delicate cells isodiametric or with the longer axis in the radial direction. A periderm is not developed, although occasionally a shorter or longer band of cork cells is noticeable immediately below the zone of spore-containing cells, or may surround in concentric layers some deep seated resting spores. The walls of the cells containing the resting spores, as well as the neighboring cells, become lignified and suberized, and since the heavy walls of the resting spores undergo like changes, a lignin stain applied to a section of the wart makes the fungus infected area discernible to the naked eye and permits of ready differentiation with the aid of the microscope.

The cortical tissue (Pl. 5), which comprises by far the largest part of the wart, is composed of simple, large parenchyma cells. The cells are crowded with starch grains. Sugar is always present in varying amounts and also tannins, primarily in newly infected regions, where, under the influence of the parasite, cellular activity is at its height. Other cell inclusions are more conspicuous by their absence. Calcium oxalate and protein crystals which are usually found in the peripheral region of the normal tuber are usually wanting. Protein crystals have been observed only in the outer cortical cells of the host—never in typical wart tissue.

Modifications of the normal type of storage cells of the wart are frequently observed and consist in their transformation into sclereids. They have been found abundantly in the aerial parts of the potato plant affected with blackleg (*2*) and normally in small numbers in the cortex of the underground stem and in the parenchyma tissue of dormant eyes.

The vascular tissue is centrally located, and only in transition region is there found a siphonostele inclosing a large pith. The relative position of xylem and phloem is, at best, only approximate. Especially where the vascular elements are greatly reduced, the phloem and xylem appear independent of each other and with no definite orientation with respect to the axis of the organ. In small branches of the wart, however, phloem groups may be observed in close proximity to the xylem and may even surround it so completely as to give the effect of a typical amphicribral bundle. While the arrangement of the vascular tissue is certainly a departure from the normal type, it must be remembered that similar, if not identical, conditions exist in normal organs, such as the tuber (*1*)

[3] Reference is made by number (italic) to "Literature cited," p. 967.

The phloem groups abut directly on the cortex. There is no endodermis; and only groups of small-celled tissue, the homolog of a pericycle, may delimit the phloem from the storage tissue. The phloem groups are small and widely scattered; sometimes a sieve tube with a single cambiform cell may constitute an entire group. Often, however, the groups are larger and attain the size of the inner phloem of the tuber. The relative number of sieve tubes and parenchyma cells is approximately that found in the phloem of the underground organs and is small compared with the aerial parts. When stained with chlorozinciodid the side walls of the sieve tubes in contact with cortical cells show a network of fine, dark-staining bands inclosing areas of lighter color. Since there is an obvious need for rapid movement of food from the storage parenchyma to the growing region of the wart, the extensive pitting of the sieve tube with these storage cells appears as a functional adaptation; the converse relation, however, may also hold.

The xylem is composed of narrow, porous elements with secondary thickenings in the form of rings and close spirals. The end walls of the cells are chiefly oblique, being sometimes, however, strictly transverse. Since the course of the vascular tissue is very irregular and since branching and anastomosing takes place frequently, the xylem cells are commonly atypical. This diversity of form finds expression in abnormal wall sculpture, excessive slope of the end walls, and a general irregular form of the element as a whole. Typical fibers and pitted vessels are wanting. Narrow parenchymatous elements of variable length often separate the xylem from the cortex. Besides being smaller, these elements differ further from typical cortical cells in being devoid of starch. The walls of the xylem elements remain cellulose for a long period. This fact and the conspicuous absence of fibers and tracheids shows that the tissue is adapted primarily for conduction and not at all for support. Such qualitative reductions in vascular tissues are common in simple galls and appear to be adaptations for changes in function. Bally (*3*) believes that the adaptation in case of the wart is for water storage rather than for conduction. It might be questioned, however, whether conduction is second in importance to water storage in wart tissue; and may we not, from morphological considerations, expect only simple protoxylem elements in a tissue as simple, as well protected and as rapidly growing as that of the wart? Both xylem and phloem cells advance independently close to the peripheral region of the wart, a fact which further tends to emphasize the necessity for rapid movement of plastic materials as well as of water. Individual sieve tubes and protoxylem cells may be observed even in small warts, which makes it difficult to tell which of the vascular elements are differentiated first and whether phloem cells or xylem cells are more important in the early growth of the organ. Although normally elements that are essentially vascular rarely terminate a bundle, in the wart tissue individual sieve tubes and also ringed protoxylem cells have been observed to advance to the periphery of the organ unaccompanied by parenchyma cells.

The older basal part of the wart and more especially the transition region show a gradual approach to the normal structure. There is a steady increase in supporting tissue and improved provision for conduction of larger quantities of water. We may then expect to find fibers and pitted vessels in increasing numbers, and indeed we do. Withal there is a complete though gradual transition from the normal to the wart tissue, and although in its simplest form the wart shows both

qualitative and quantitative reduction of tissues, there exists, nevertheless, a marked similarity between structure of normal host and wart, and this is shown not only in similarity of position of the tissues with reference to each other but also in their practical identity as regards the essential composition of the vascular tissue.

MORPHOLOGICAL NATURE OF THE WART

From anatomical analysis the wart is a homoplastic growth with quantitative reduction in vascular tissue and increase in storage parenchyma. The likeness is even more marked and reaches a point of complete identity if we choose to compare reduced host tissue with transition regions in the wart. But even if we accept this conclusion, there still remains the answer to the question as to the nature of the wart in its entirety and its relation to the normal organs of the plant. To arrive at a satisfactory conclusion it will be necessary to follow briefly the early ontogeny of the wart and to consider the nature of the stimuli which initiate the abnormal growth.

A newly infected bud shows the surface covered with small pustules which in sectional view (Pl. 4, A) are found to consist of a small-celled tissue rich in protoplasm and very turgid. Certain of these protuberances show a slight depression with a brownish center in which is found a prosorus or summer sporangium of the parasite. These areas of new growth, according to Curtis (*4*) and Bally (*3*), have resulted from the stimulating effect of the fungus. Since the epidermal walls of these pustules are very delicate, and since new summer spores are formed and mature in rapid succession, new infections occur, and as a consequence new centers of growth are formed in direct proportion to the number of new infections. The finely adjusted stimulation which results in the formation of a progressively increasing area of meristematic tissue permits of an uninterrupted development of the parasite and the formation of large numbers of resting spores which under suitable conditions remain viable in the soil and form a latent source of infection for years to come.

In semiresistant varieties wart development remains superficial. The growth slightly resembles scab pustules, though the infected areas are somewhat more elevated (Pl. 3, C, D). In susceptible varieties, on the other hand, the warts are extensive structures (Pl. 2, A, D, C, E; 3 A, B) and only in their topography bear resemblance to the former kind. Percival (*8*) assumes that the wart is "a malformed branch system stimulated by the parasite to grow irregularly and before its natural time." A study of Plate 3, B, suggests no objection to such a theory, and a consideration of the anatomy of the vascular system seems only to lend additional support. However, granting that the stimulating effect of the fungus may result in a shortening or complete elimination of the rest period of the young bud, may we not expect a similar phenomenon in potato plants which are semiresistant to the wart? Yet this is not the case. Furthermore, an analogy with "witches'-broom" formation in Pteris (*5*) indicates that it may be unessential, however, to presuppose the existence of buds in order to initiate growth of extensive though somewhat abnormal foliar organs; that, in fact, the stimulus exerted by the fungus is sufficient to bring about cell division and initiate organ formation in meristematic regions of plants. In the potato, too, extensive wart formation often results from the infection of an outer bud scale which has normally completed development. The association

of the fungus with reactive host cells is sufficient to initiate new growth. But the same parasite in the subsequent development of the new organ acts as inhibitor and modifier and thus alters the appearance of the new structure completely. Early developmental studies and the comparative ease with which the pathogene is identified have left no doubt that the wart, as a whole, is normal host tissue and that only the peripheral regions contain typical traumatic cells. An analogous case is presented by Dr. E. F. Smith (*9*). The growth resulting from inoculation is composed of normal host tissue and not of parasitized cells, as viewed by Levine (7). The present impossibility of demonstrating the crowngall pathogene in the tissues, however, gives only presumptive evidence, but a consideration of the reaction of host and parasite in case of the wart of potato should give, if analogy be worth anything, a strong support to the theory defended by Doctor Smith.

The wart can be considered a foliar branch system. The existence of various types of warts seems to be bound up with the relative susceptibility of the host, the nature and extent of the primary infection, and such factors as tend to modify plant growth in general.

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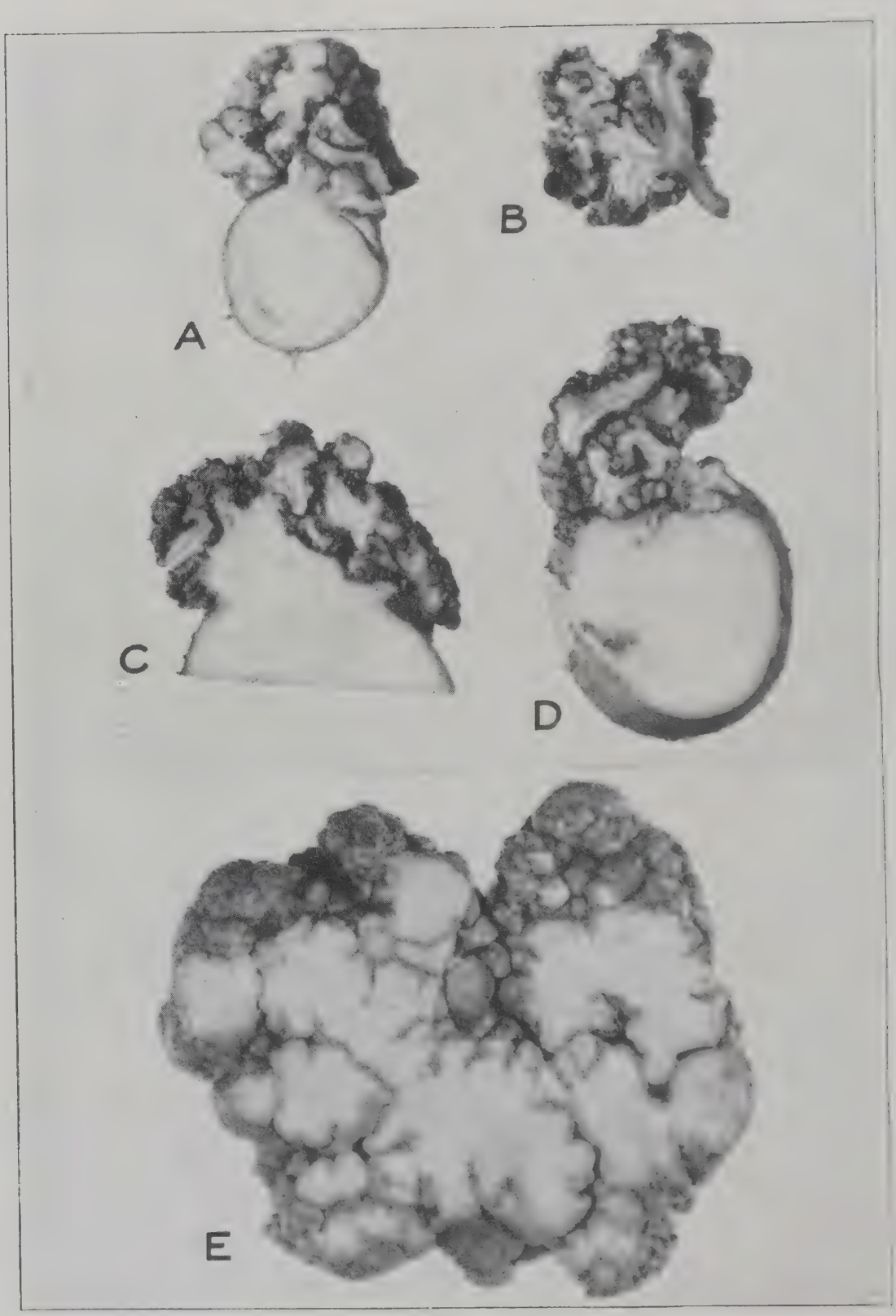
PLATE 1

Color photograph showing wart infection on underground stalk, stolons, and tubers. (Photograph by Freeman Weiss.)

Journal of Agricultural Research Washington, D. C.


A
B
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D
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PLATE 2

A.—Sectional view of large wart developed from terminal bud of tuber.

B.—Sectional view of aerial wart. Note general appearance of wart and central location of vascular tissue.

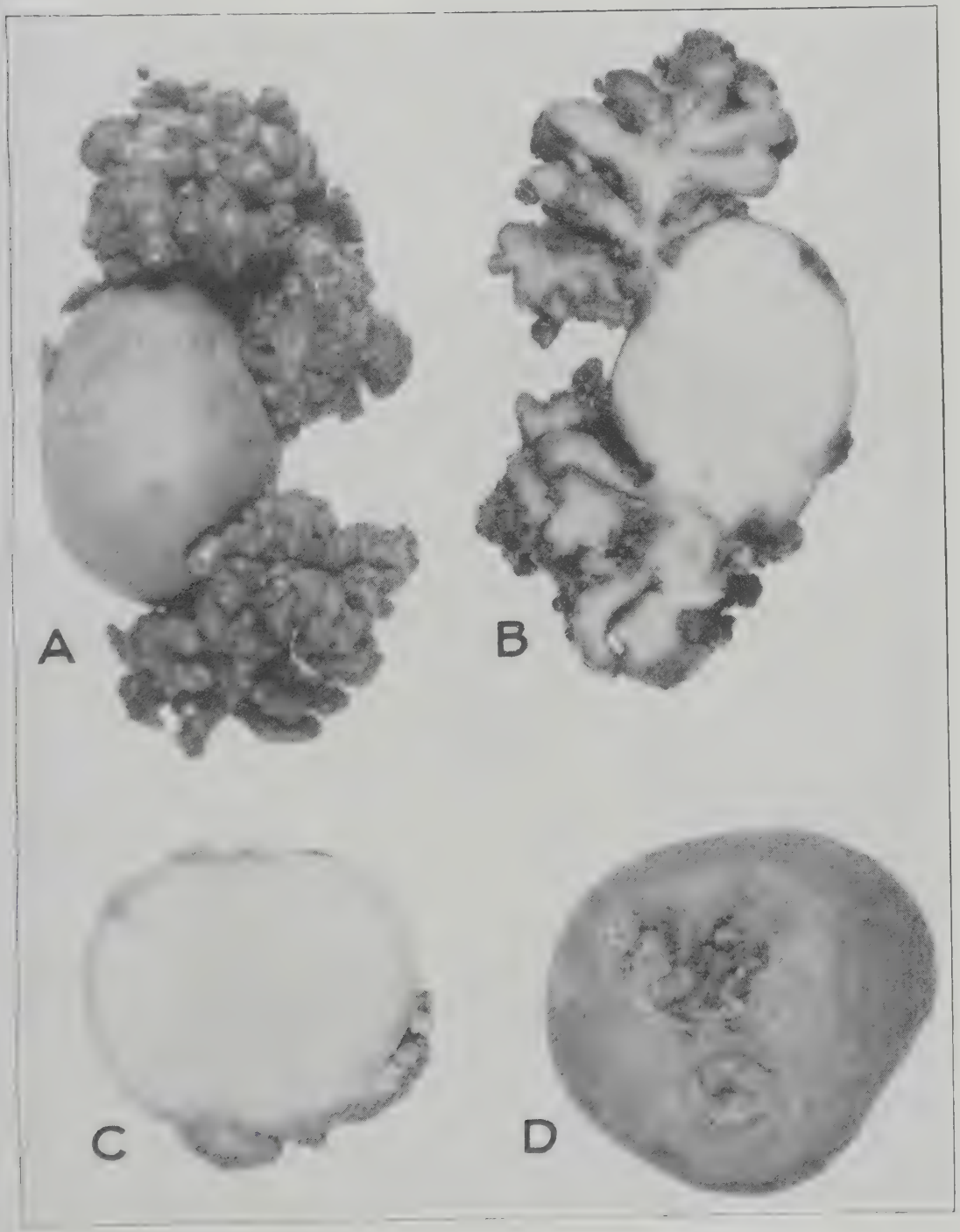
C.—Sectional view of large wart on tuber. The vascular tissue shows the arrangement which is typical of petiolar or midrib bundles.

D.—Sectional view of large wart on tuber. The apparent solid structure is an aggregate of compressed, modified leaves.

E.—Sectional view of large wart on stolon. The branching is often so intricate that only the aid of the microscope will reveal the component parts.

PLATE 3

A.—Surface view of large wart on tuber.
B.—Sectional view of the same wart, showing characteristic branching.
C.—Sectional view of wart on a semiresistant variety.
D.—Surface view of the same wart.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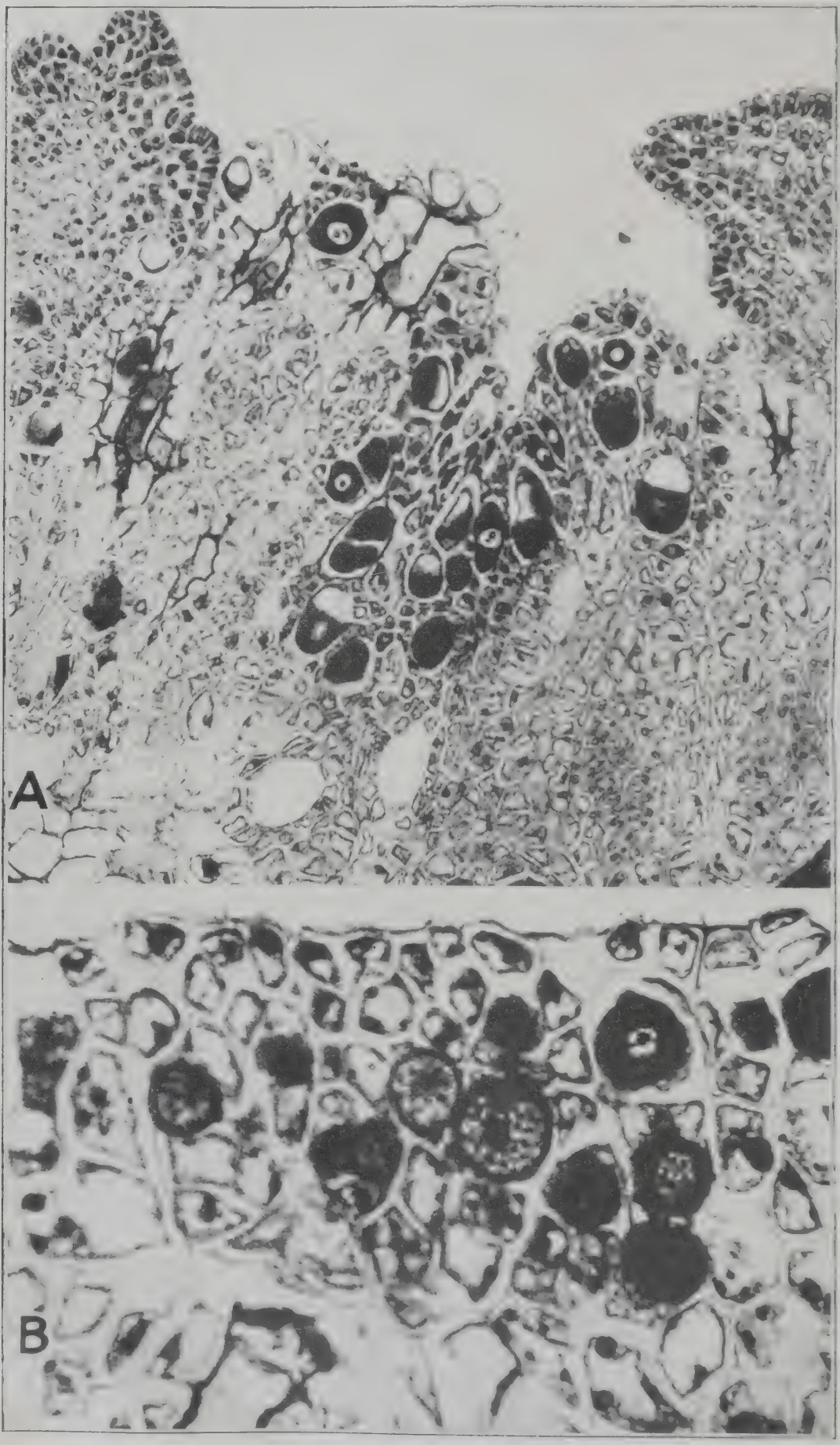
A
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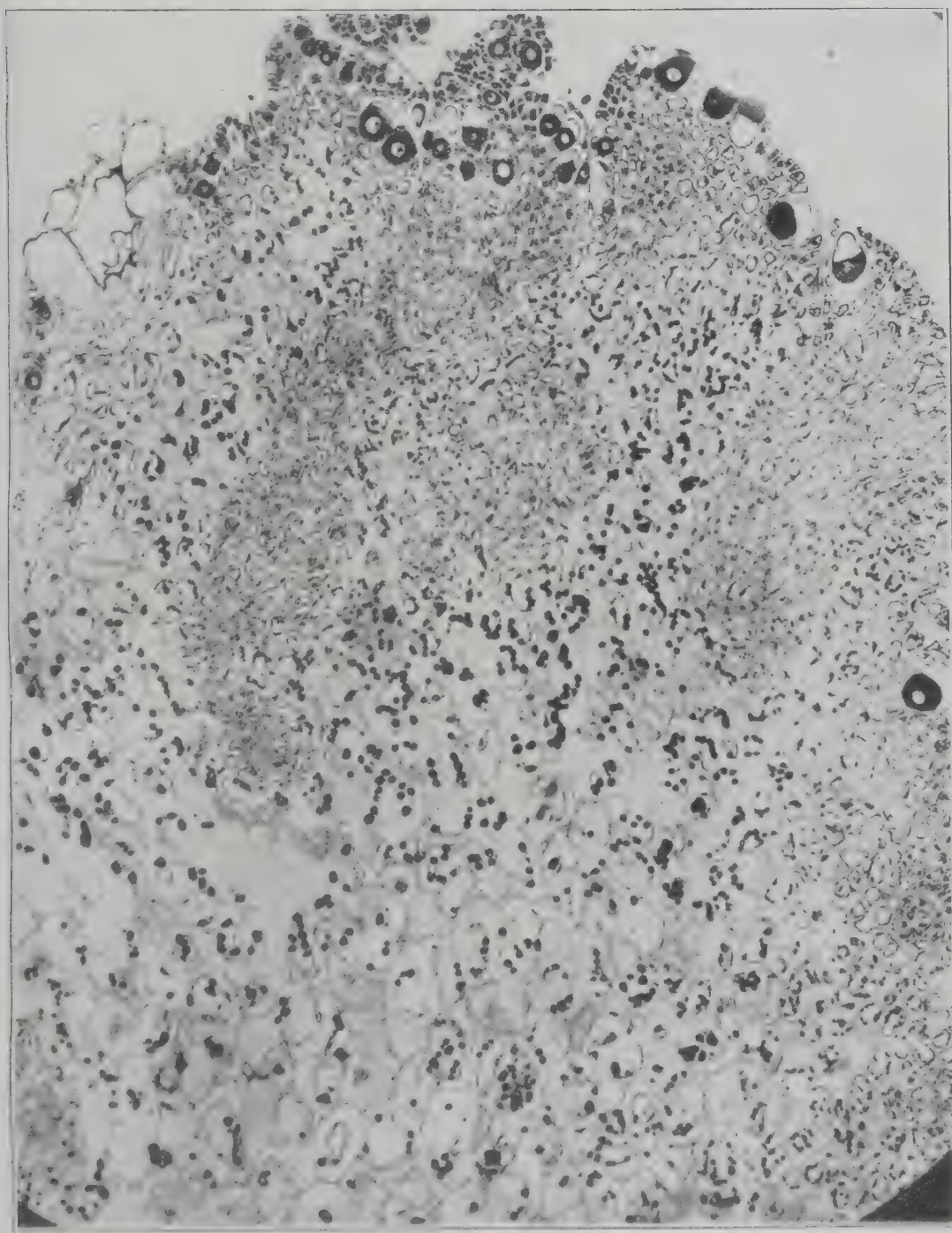
PLATE 4

A.—Section through the peripheral region of young wart, showing resting spores, summer sori, and new centers of growth which have resulted from the stimulating effect of the parasite.

B.—Section though an older part of a wart, showing structure and position of resting spores.

PLATE 5

Section of young wart, showing general appearance of tissues and location of spore-bearing cells.



INFLUENCE OF TEMPERATURE AND EVAPORATION UPON THE DEVELOPMENT OF APHIS POMI DEGEER[1]

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INTRODUCTION

Entomologists attacking economic problems are ever impressed with the need of a more thorough knowledge of the influence of the environmental factors which control the activities of the insects under investigation. The interrelations between insects and their environment are highly complex, and attempts at their determination are fraught with many difficulties. That any effort in this direction is well worthy of the expense in time and energy there can be little doubt, for, while this is as yet a comparatively unexplored field, there are many instances of the application of a knowledge of environmental factors to the applied control of economic pests. Headlee [3] pointed out the possibilities of the reduction of certain stored grain pests by modifying the relative humidity of the air within the container. Lovett and Fulton,[4] in discussing the control of the codling moth in the Willamette Valley, state:

> Where the evening temperature during May at 8:00 p. m. is 60° or above, the first generation codling moths may be expected to deposit eggs. Consequently when this temperature of 60° at 8:00 p. m. is registered, it is the proper time to apply the "thirty-day" codling moth spray.

A realization of the economic bearing of such information prompted the writer to make observations of certain climatic factors in connection with a study of the activities of several species of apple aphids, a group of insects which seem especially susceptible to climatic influence. A considerable amount of data on evaporation, temperature, and precipitation has been collected which, aside from the bearing upon the present study, should be of considerable general interest to both plant and animal ecologists.

In order to concentrate the study and to avoid scattering the data over too broad a field, observations in this connection were practically limited to a single species *Aphis pomi* DeGeer.

An understanding of the fundamentals of the life history of the species is desirable in considering the data here presented. For this reason the seasonal cycle is briefly reviewed.

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[1] Accepted for publication Jan. 16, 1922.

[2] The writer is indebted to Prof. A. L. Lovett for encouragement in the prosecution of this study, and to Mr. Richard Jones whose painstaking work as Technician in the Department of Entomology relieved the writer of much of the routine work of this study.

[3] HEADLEE, Thomas J. SOME FACTS RELATIVE TO THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON INSECT METABOLISM. *In* Jour. Econ. Ent., v. 10, no. 1, p. 31–38. 1917.

[4] LOVETT, A. L., and FULTON, B. B. FRUIT GROWER'S HANDBOOK OF APPLE AND PEAR INSECTS. Oreg. Agr. Exp. Sta. Circ. 22, p. 8. 1920.

LIFE HISTORY OF APHIS POMI

The green apple aphis passes the winter in the egg stage. The eggs are placed on the bark of the water sprouts and other terminal growths of apple and frequently occur grouped in immense numbers. With the unfolding of the buds in the spring, these eggs hatch, and the tiny nymphs migrate to the newly developed foliage.

These "stem mothers" and the succeeding generations feed upon the succulent foliage of apple throughout the summer. There is no migration to alternate food plants as there is with many species of aphids, and the winged forms which are produced serve merely to disseminate the species.

Reproduction throughout spring and summer is entirely viviparous and parthenogenetic. With the approach of fall, males and oviparous females are produced, and the over-wintering eggs are deposited.

There are a variable number of generations during the season, depending upon environmental conditions.

METHODS EMPLOYED

This work has been entirely in the nature of a field study, and no attempt was made to modify or control the conditions of temperature or moisture.

The experimental plot was located on the college farm at Corvallis, Oreg., about one mile from the Agricultural Building and had an elevation of approximately 225 feet above sea level. This plot consisted of 1- and 2-year-old Greening apple trees planted in two rows about 4 feet apart with the trees about 3 feet apart in the rows. To the westward about 60 to 75 feet distant there was a dense growth of alders along the banks of Oak Creek. These trees, being some 35 feet in height, served to break the force of the strong, westerly "sea breezes" prevalent during the summer months.

As the aphid eggs hatched, numbers of the nymphs were transferred to suitable buds on the experimental trees and here allowed to mature. In obtaining nymphs of the later generations, a number of adults would be placed on a suitable aphis-free leaf cluster. The following day these adults were removed, and the nymphs born during the 24-hour period were allowed to remain. These nymphs were permitted to mature in order to obtain the length of the developmental period. The developmental period was reckoned from the day after birth till the day of the appearance of the first young, inclusive.

The trees upon which the experiments were being conducted were protected by a special type of cage (fig. 1). This consisted of a cylinder of galvanized wire cloth, open at one end. To the open end of this cage was attached a cheesecloth skirt. The cage was inverted over the tree and held in position at the proper height by means of a stake set near by. The cheesecloth skirt was gathered about the trunk of the tree and tied with a cord. A band of cotton batting placed at the proper height on the tree rendered a perfect fit between the cloth skirt and the tree, and prevented binding of the trunk from the tie-cord. This type of cage is easily removed for examination of the aphids, is as readily replaced, and has proved quite satisfactory for this work.

The evaporation records were obtained by means of a "nonabsorbing" evaporimeter or atmometer (fig. 2) similar to instruments used in many evaporation studies by various workers during recent years. Standardized, spherical, porous, porcelain cups were obtained from the "Plant

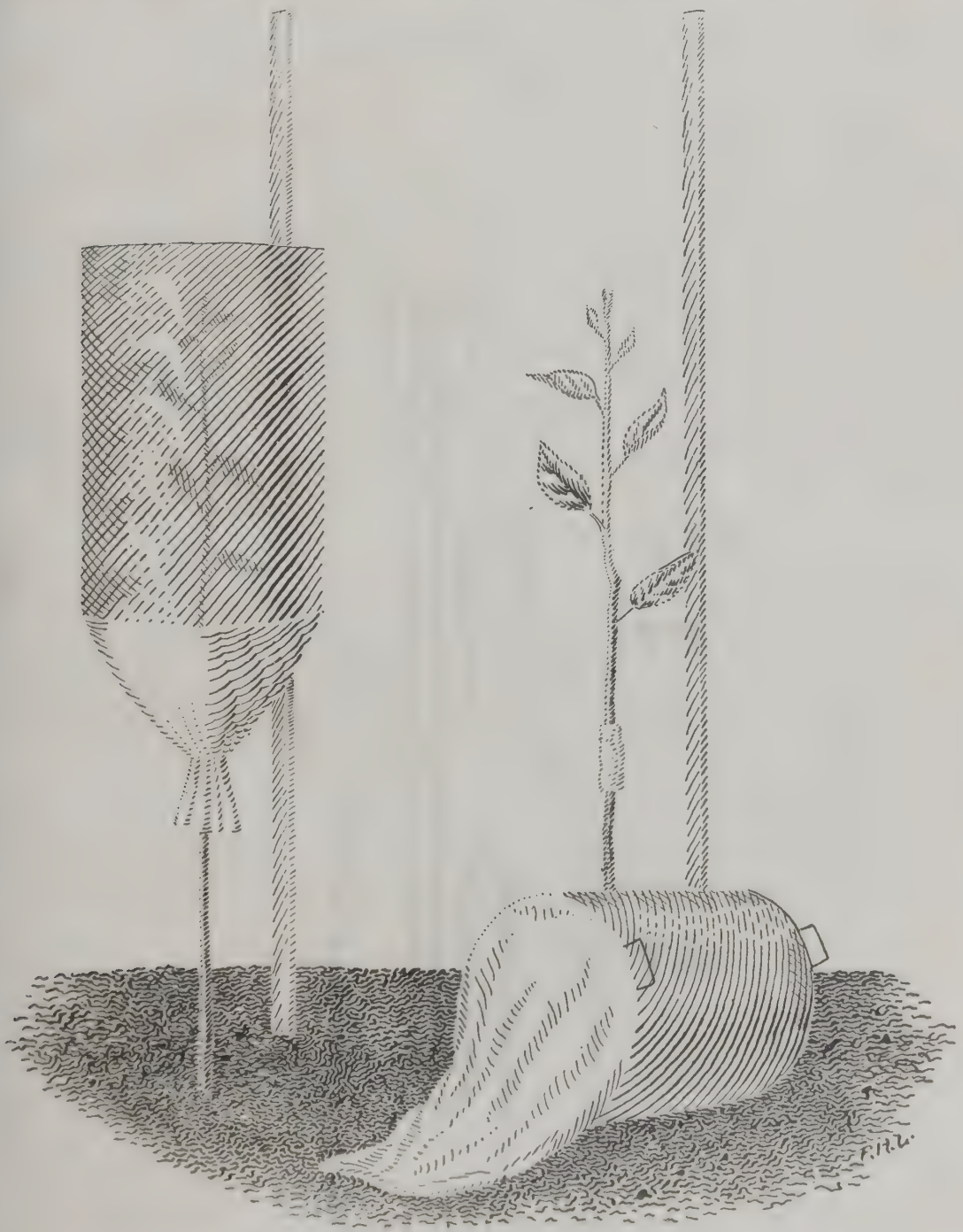

FIG. 1.—Cage used in rearing aphids.

World," and results as here given should be comparable with results obtained elsewhere [5] by means of similar instruments.

Observations were made daily at 9.30 a. m. The amount of evaporation was determined by filling the evaporimeter at this time.

[5] LIVINGSTON, Burton Edward. ATMOSPHERIC INFLUENCE ON EVAPORATION AND ITS DIRECT MEASUREMENT. In Mo. Weather Rev., v. 43, no. 3, p. 126–131, 2 fig. 1915. References and notes, p. 131.

A graduated pipette was used and readings were made to tenths of cubic centimeters.

Temperature records were obtained by means of a Tycos Dial Type Mercury Recording Thermometer, manufactured by Taylor Instrument

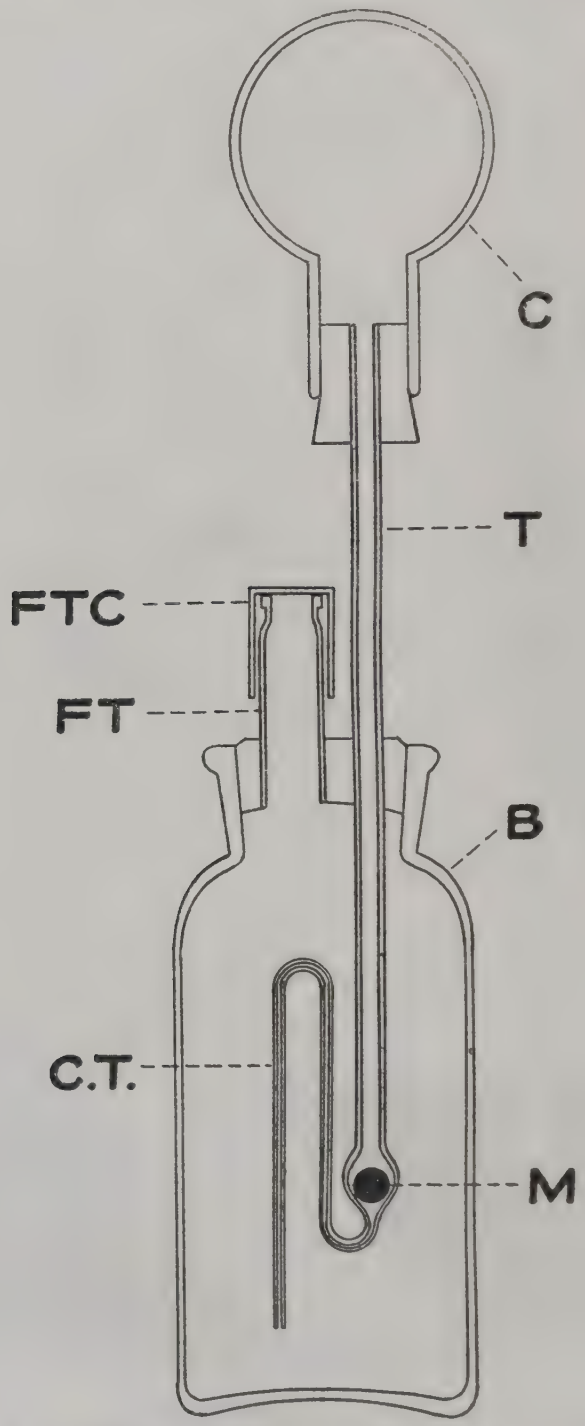

FIG. 2.—Sectional view of atmometer: C, standard, spherical, porous, porcelain cup; T, water supply tube; B, water bottle; FT, filling tube; FTC, filling tube cover; M, mercury drop to prevent return of water from cup; CT, capillary tube.

Companies, Rochester, N. Y. The daily mean temperature was calculated by reading the temperatures on the charts for each half hour and averaging these 48 readings. The mean temperature for any given period of days was obtained by averaging the daily mean temperatures.

For the records of precipitation, the writer is indebted to the Department of Soils of the Oregon Agricultural Experiment Station. Observations on precipitation were made daily at 5.00 p. m. and hence are not exactly comparable with the temperature and evaporation records as given in this paper.

TABLE I.—*Daily evaporation and precipitation, 1919, at Corvallis, Oreg.*

Date.	Evaporation.	Precipitation.	Character of weather.	Date.	Evaporation.	Precipitation.	Character of weather.
	Cc.	*Inches.*			*Cc.*	*Inches.*	
Mar. 31	22.5	0		May 21	.....	0	
Apr. 1	11.2	0	Cloudy.	22	49.1	0	
2	.....	0	do.	23	22.8	0	
3	13.0	0.15	do.	24	.....	0	Partly cloudy.
4	5.7	.65	do.	25	16.4	.55	Cloudy.
5	7.0	.57	do.	26	13.3	0	Do.
6	8.0	.04	do.	27	14.7	0	Do.
7	10.3	0	Partly cloudy.	28	18.0	0	Do.
8	12.7	0	do.	29	9.3	.15	Partly cloudy.
9	.....	0	do.	30	16.1	.06	Do.
10	24.0	.20	do.	31	.....	0	
11	.....	.08		June 1	61.4	0	
12	24.0	0		2	27.7	0	
13	13.8	.12	do.	3	34.0	0	
14	18.8	0		4	45.6	0	
15	14.9	0		5	31.3	0	
16	1.7	.12	Cloudy.	6	30.3	0	
17	4.1	.71	do.	7	.....	0	
18	6.5	.47	do.	8	58.7	0	
19	.....	.15	do.	9	14.0	.02	
20	18.7	.10	Partly cloudy.	10	10.9	0	Do.
21	.....	0		11	13.7	0	Do.
22	34.2	0		12	12.9	0	Do.
23	14.9	0		13	9.5	.08	
24	12.2	T.	do.	14	16.3	0	Do.
25	8.4	.07	do.	15	12.3	.02	
26	.....	0		16	18.5	0	Do.
27	31.8	0		17	28.6	0	
28	18.4	0		18	27.9	0	
29	26.4	0		19	23.2	0	Do.
30	34.6	0		20	15.4	0	Do.
May 1	19.7	0		21	21.3	0	Do.
2	17.9	0	do.	22	17.2	0	Do.
3	.....	0		23	27.2	0	
4	91.7	0		24	29.4	0	
5	35.6	0		25	30.9	0	
6	39.1	0		26	8.8	.10	
7	26.1	0		27	21.9	0	Do.
8	17.7	0	do.	28	17.0	0	Cloudy.
9	20.2	0	do.	29	29.5	0	
10	.....	0	do.	30	24.7	0	
11	24.7	.11	do.	July 1	25.6	0	
12	9.1	.10	do.	2	33.5	0	
13	24.6			3	39.1	0	
14	8.8	.05	do.	4	28.4	0	
15	7.9	.05	Cloudy.	5	20.2	0	Partly cloudy.
16	5.3	.22	do.	6	16.9	0	Do.
17	.....	0	do.	7	38.7	0	
18	21.4	0.03	do.	8	47.3	0	
19	21.4	0		9	41.6	0	Do.
20	31.0	0		10	22.0	0.2	

TABLE I.—*Daily evaporation and precipitation, 1919, at Corvallis, Oreg.*—Continued

Date.		Evaporation.	Precipitation.	Character of weather.
		Cc.	*Inches.*	
May	11	18.4	0	do.
	12	36.5	0	
	13	52.4	0	
	14	44.2	0	
	15	32.5	0	
	16	34.8	0	
	17	35.5	0	
	18	40.2	0	
	19	51.3	0	
	20	47.5	0	
	21	54.0	0	
	22	58.5	0	
	23	17.5	.08	
	24	23.9	0	do.
	25	16.3	0	
	26	.....	0	
	27	54.0	0	
	28	22.4	0	
	29	9.0	0	Cloudy.
	30	20.5	0	Partly cloudy.
	31	15.9	0	do.
Aug.	1	14.9	0	do.
	2	11.3	0	do.
	3	9.0	0	do.
	4	14.6	0	do.
	5	31.5	0	do.
	6	27.3	0	do.
	7	17.9	0	do.
	8	25.0	0	
	9	33.5	0	
	10	34.1	0	
	11	18.0	.05	Partly cloudy.
	12	16.1	0	do.
	13	36.5	0	
	14	59.0	0	
	15	47.3	0	
	16	21.7	0	
	17	13.9	0	do.
	18	25.5	0	
	19	34.6	0	
	20	34.2	0	
Aug.	21	32.3	0	
	22	35.8	0	
	23	39.2	0	
	24	32.5	0	
	25	28.7	0	
	26	18.3	0	Partly cloudy.
	27	31.3	0	
	28	39.1	0	
	29	28.7	0	
	30	15.6	0	Do.
	31	11.7	.09	Do.
Sept.	1	29.8	0	
	2	22.0	0	
	3	3.9	T.	Do.
	4	3.1	.40	Cloudy.
	5	0.7	.70	Do.
	6	.....	.25	Do.
	7	7.8	.01	Partly cloudy.
	8	0.6	.25	Cloudy.
	9	8.7	0	Partly cloudy.
	10	13.9	0	
	11	2.7	.40	Cloudy.
	12	22.0	0	
	13	46.0	0	
	14	27.8	0	
	15	16.1	0	Do.
	16	6.5	0	Do.
	17	10.9	0	Do.
	18	19.4	0	
	19	17.6	0	
	20	28.2	0	
	21	78.6	0	
	22	43.8	0	
	23	28.8	0	
	24	22.3	0	
	25	19.8	0	
	26	17.0	0	
	27	.....	.12	Do.
	28	19.7	.4	
	29	.....	0	
	30	6.3	.55	Do.

TABLE II.—*Daily temperature, evaporation, and precipitation at Corvallis, Oreg., 1920*

Date.		Temperature.			Evaporation.	Precipitation.	Character of weather.
		Minimum.	Mean.	Maximum.			
		° *F.*	° *F.*	° *F.*	*Cc.*	*Inches.*	
Mar.	29.....	35	45.6	58	12.6	0	
	30.....	24	34.0	48	7.1	0.50	Cloudy.
	31.....	33	38.6	47	5.9	.32	Do.
Apr.	1.....	39	41.8	46	10.2	.20	Do.
	2.....	34	41.1	51	20.4	0	Partly cloudy.
	3.....	45	48.2	62	.....	.04	Cloudy.
	4.....	44	49.4	62	23.0	.03	Partly cloudy.

TABLE II.—*Daily temperature, evaporation, and precipitation at Corvallis, Oreg., 1920*—Continued

Date.	Temperature.			Evaporation.	Precipitation.	Character of weather.
	Minimum.	Mean.	Maximum.			
	° F.	° F.	° F.	Cc.	Inches.	
Apr. 5	36	44.2	56	.....	0.08	
6	44	47.3	55	32.2	.03	Cloudy.
7	33	41.5	54	9.2	.08	Do.
8	32	40.2	50	3.6	.58	Cloudy.
9	40	44.3	55	.....	.10	Do.
10	29	45.6	60	.....	.10	
11	42	50.6	65	33.6	0	
12	37	45.2	54	3.3	.12	
13	37	43.7	56	7.9	.11	Partly cloudy.
14	41	45.5	56	6.7	.35	Cloudy.
15	29	41.9	56	12.4	.10	
16	30	42.4	52	12.3	.05	Partly cloudy.
17	27	42.7	56	.....	.01	
18	42	51.1	64	27.9	0	
19	30	39.7	56	7.9	.24	Cloudy.
20	28	39.0	48	6.0	.11	Partly cloudy.
21	30	39.0	52	5.2	.06	Do.
22	26	39.9	57	.....	.09	Do.
23	28	43.9	59	19.5	0	
24	31	49.2	63	.....	0	
25	32	54.8	70	42.2	0	
26	36	57.5	78	26.3	0	
27	39	51.7	68	.....	0	
28	40	48.5	65	38.3	0	
29	38	46.3	55	13.7	0	
30	37	46.5	59	15.2	.01	
May 1	36	46.1	60	.....	0	
2	30	47.5	62	34.0	0	
3	30	48.4	64	17.3	0	
4	31	49.3	64	19.1	0	
5	33	52.6	69	19.0	0	
6	37	58.5	79	24.4	0	
7	46	56.7	78	22.4	0	
8	40	53.0	69	.....	.03	
9	27	46.9	66	45.0	0	
10	28	46.6	64	20.8	0	
11	30	48.0	64	21.8	0	
12	30	50.3	68	25.9	0	
13	28	49.0	69	24.3	0	
14	38	55.9	72	33.9	0	
15	35	55.1	73	.....	0	
16	50	61.6	80	56.4	0	
17	37	53.5	70	15.9	.23	
18	31	51.5	74	24.2	0	
19	35	52.2	70	25.1	0	
20	34	48.9	66	11.9	0	
21	27	47.5	64	20.4	0	
22	42	52.1	65	.....	0	
23	34	48.1	65	33.6	0	Do.
24	25	42.8	59	13.2	0	Do.
25	32	50.9	66	21.5	0	
26	37	52.1	68	15.5	0	Do.
27	34	50.1	69	20.0	.17	
28	34	49.6	68	17.8	.01	
29	26	44.3	61	.....	.05	Do.
30	28	47.0	64	31.3	.02	
31	30	52.7	70	31.7	0	
June 1	35	58.4	78	40.2	0	
2	44	64.1	85	39.1	0	

TABLE II.—*Daily temperature, evaporation, and precipitation at Corvallis, Oreg., 1920–* Continued

Date.	Temperature.			Evaporation.	Precipitation.	Character of weather.
	Minimum.	Mean.	Maximum.			
	° *F.*	° *F.*	° *F.*	*Cc.*	*Inches.*	
June 3	47	63.3	88	44.6	0	
4	42	58.6	79	24.7	0	
5	45	58.4	75	.....	0	
6	51	53.5	63	23.4	.10	Cloudy.
7	45	52.0	58	5.1	.41	
8	37	52.4	69	12.7	.39	Partly cloudy.
9	38	53.5	72	21.9	0	
10	50	55.5	66	10.5	0	Cloudy.
11	45	54.6	66	16.9	.03	Partly cloudy.
12	49	57.4	74	.....	0	
13	54	57.5	62	20.1	.20	Cloudy.
14	44	55.8	67	22.3	.53	Partly cloudy.
15	36	52.0	68	15.2	0	Do.
16	52	58.2	72	14.1	0	Do.
17	38	54.5	71	20.0	.19	Do.
18	39	56.8	71	27.6	0	
19	44	63.2	80	.....	0	
20	46	64.5	84	70.1	0	
21	46	59.9	82	28.8	0	
22	33	52.9	62	17.2	0	
23	35	52.2	68	26.6	0	Do.
24	42	53.0	70	16.6	0	
25	39	52.7	67	18.2	0	
26	45	62.4	78	.....	0	
27	47	64.7	82	69.6	0	
28	44	64.7	84	26.9	0	
29	49	67.7	89	34.2	0	Do.
30	43	65.3	88	28.8	0	
July 1	45	67.1	88	38.6	0	
2	46	67.3	90	43.3	0	
3	38	58.1	80	.....	0	
4	36	59.8	82	.....	0	
5	39	62.4	84	93.5	0	
6	48	68.5	89	36.5	0	
7	50	68.1	93	38.7	0	
8	43	60.0	78	23.4	0	
9	39	63.1	85	28.7	0	
10	49	60.6	80	.....	0	
11	50	57.2	68	39.9	0	Cloudy.
12	51	56.0	66	7.7	0	Do.
13	52	57.0	62	5.2	.41	
14	48	60.8	76	9.2	Trace	
15	49	64.8	86	22.8	0	Do.
16	56	64.7	88	23.7	0	Do.
17	45	63.5	81	.....	0	Do.
18	53	64.4	81	58.2	0	Partly cloudy.
19	50	61.2	74	17.9	0	Do.
20	50	64.1	82	25.0	0	
21	41	59.2	78	21.2	0	
22	41	62.9	82	30.2	0	
23	42	60.9	80	28.6	0	
24	40	59.4	77	.....	0	
25	40	63.4	84	65.8	0	
26	49	67.9	88	36.4	0	
27	54	66.0	84	35.7	0	
28	56	60.6	70	12.8	0	Cloudy.
29	40	57.6	70	13.8	0	Do.

TABLE II.—*Daily temperature, evaporation, and precipitation at Corvallis, Oreg., 1920*—Continued.

Date.	Temperature.			Evaporation.	Precipitation.	Character of weather.
	Minimum.	Mean.	Maximum.			
	°F.	°F	°F	Cc.	Inches.	
July 30	44	63.8	83	26.3	0	
31	40	61.9	80	.....	0	
Aug. 1	46	64.9	87	63.0	0	
2	45	63.8	84	28.5	0	
3	46	66.6	82	36.3	0	
4	54	69.4	89	36.6	0	
5	53	66.4	84	21.6	0	Cloudy.
6	47	65.8	88	27.7	.05	
7	59	71.6	88	.....	0	
8	56	65.6	82	53.3	0	
9	45	63.7	82	27.6	0	
10	48	67.2	88	30.9	0	
11	50	74.5	92	50.9	0	
12	53	76.9	100	49.5	0	
13	47	74.5	100	40.9	0	
14	43	68.4	97	.....	0	
15	45	67.2	93	72.9	0	
16	40	61.6	86	32.1	0	
17	32	54.0	75	23.7	0	
18	42	58.4	76	29.6	0	
19	41	67.6	88	47.4	0	
20	42	67.6	96	36.4	0	
21	46	67.0	94	.....	0	
22	42	60.7	85	52.4	0	
23	56	67.9	87	33.3	0	
24	48	61.3	77	22.6	0	Do.
25	36	51.9	70	.....	.01	Do.
26	43	58.0	76	32.5	0	
27	40	50.7	64	3.8	.22	Do.
28	51	57.8	74	.....	.02	Do.
29	30	48.2	67	27.9	.49	Do.
30	37	54.9	74	23.0	0	
31	40	65.3	85	46.4	0	
Sept. 1	41	64.6	90	32.4	0	
2	40	62.1	87	24.3	0	
3	40	58.9	82	20.1	0	
4	50	60.6	78	.....	0	
5	33	53.3	71	33.9	0	
6	32	51.4	70	19.7	0	
7	41	55.4	74	16.3	0	
8	47	53.3	62	9.7	0	Do.
9	48	51.5	58	5.0	.08	Do.
10	52	56.4	60	3.3	.23	Do.
11	49	56.9	69	.....	.01	Do.
12	48	54.2	68	14.2	1.18	Partly cloudy.
13	44	50.5	58	1.7	.95	Cloudy.
14	37	50.9	69	9.6	.26	Partly cloudy.
15	41	55.7	75	15.2	0	

EVAPORATION STUDIES

During the summer of 1919 a series of experiments was conducted in the hope that the measurement of atmospheric evaporation—combining, as it does, effects of both temperature and humidity—might, under normal outdoor conditions, give a fairly accurate index to the rate of metabolism of *Aphis pomi*. The study was continued during the summer of 1920.

The daily evaporation rates during the periods covered by these inves tigations are given in Tables I and II and are shown graphically i figures 3, 4, 5, and 6.

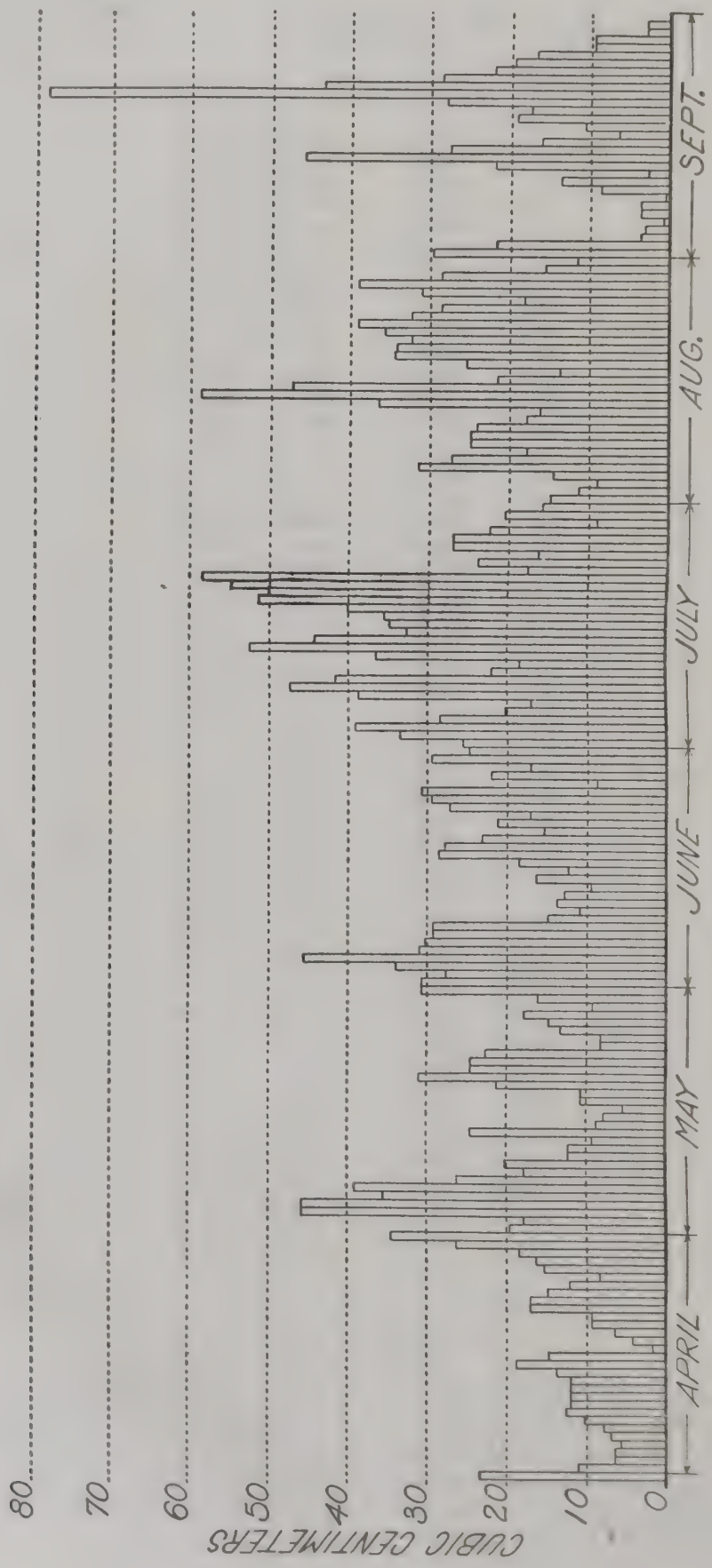

FIG. 3.—Daily records of evaporation, Corvallis, Oreg., summer of 1919.

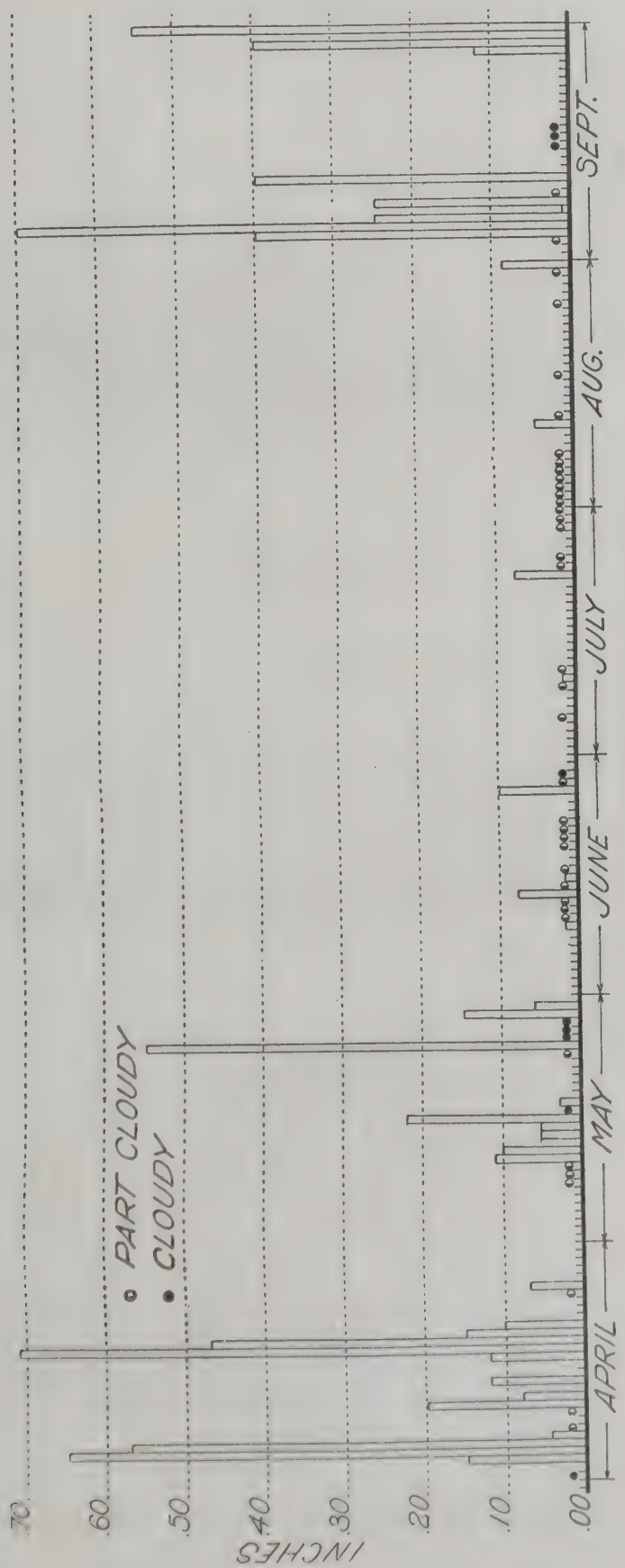

FIG. 4.—Daily records of precipitation, Corvallis, Oreg., summer of 1919.

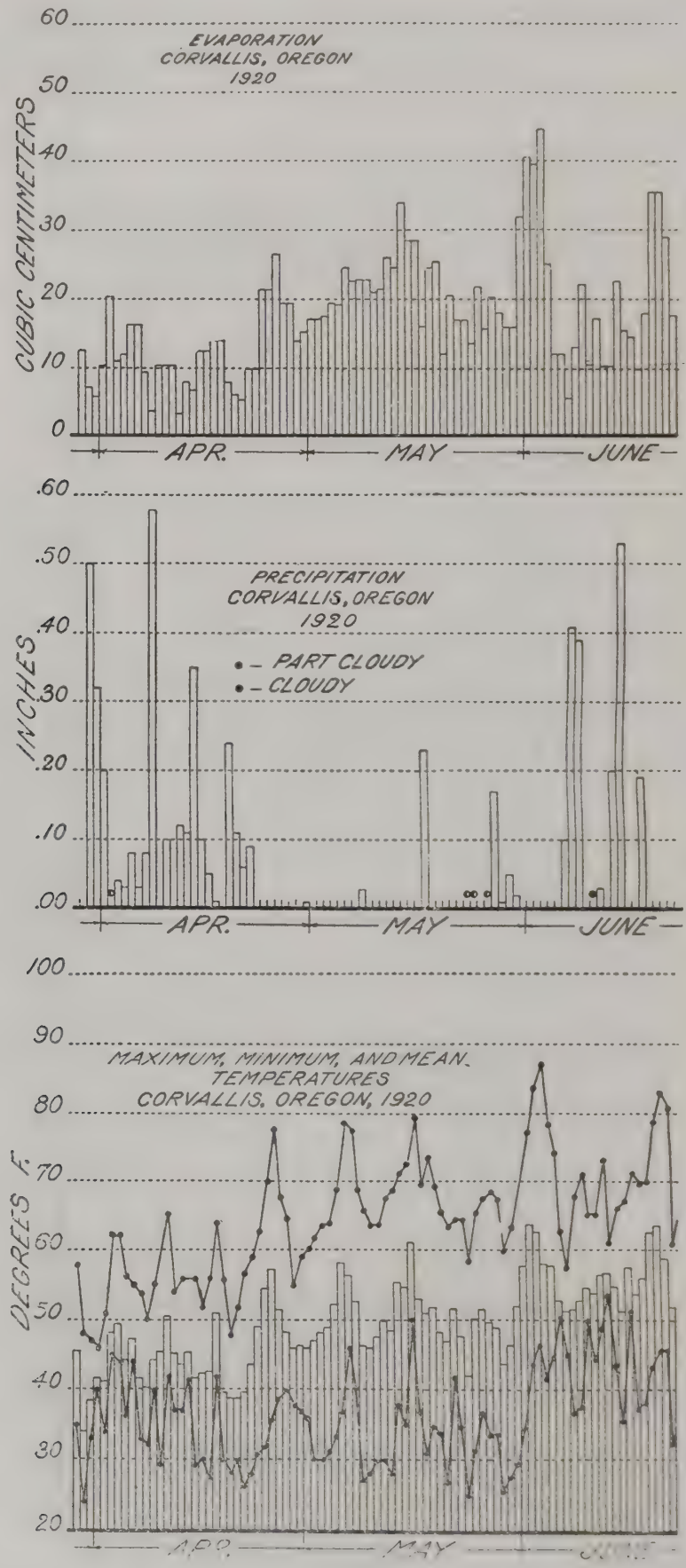

FIG. 5.—Daily records of evaporation, precipitation, and temperature, Corvallis, Oreg., summer of 1920.

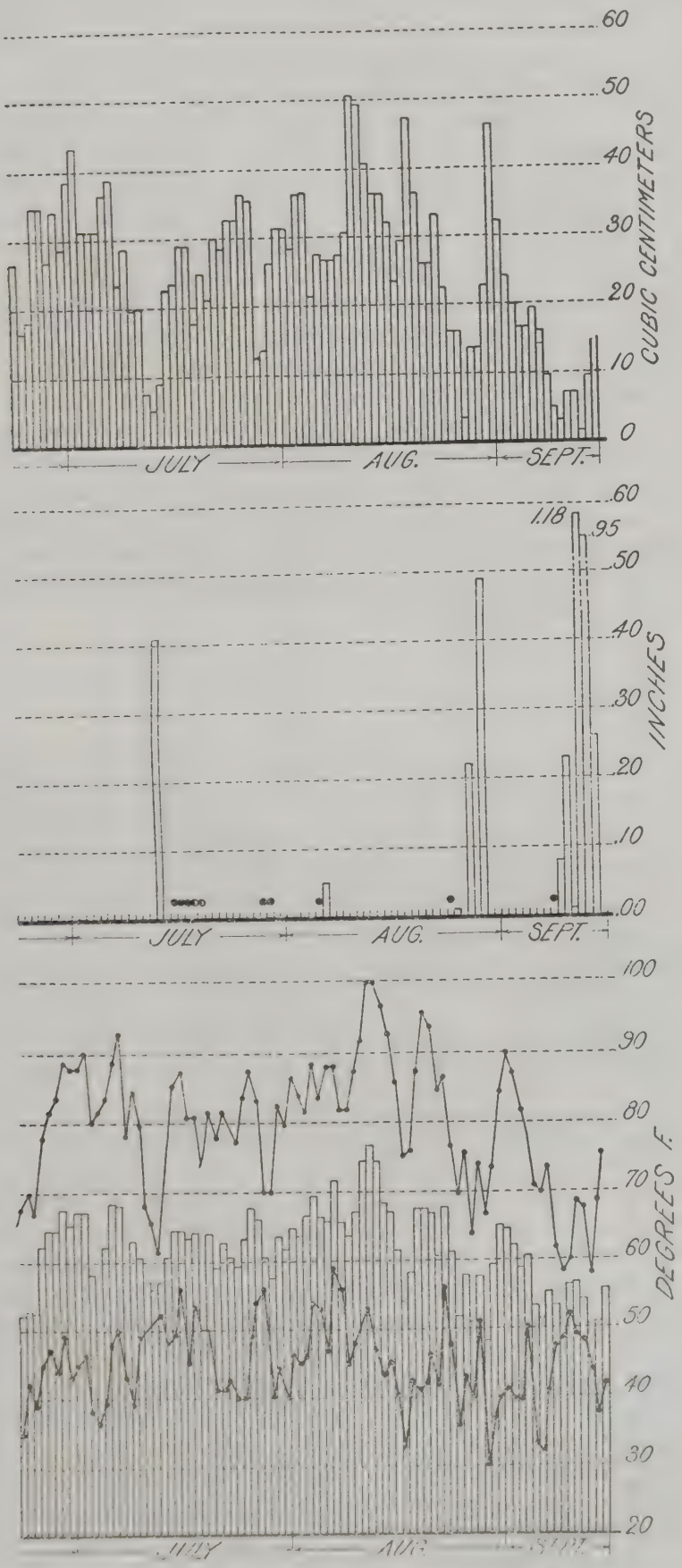

FIG. 6.—Daily records of evaporation, precipitation, and temperature, Corvallis, Oreg., summer of 1920.

During the summer of 1919 the highest daily evaporation of 78.6 c occurred on September 21. This was probably due to continuous wi movement rather than to unusually high temperature or low humidit The highest evaporation recorded during 1920 occurred on August being 50.9 cc. As would be expected, there is a marked correlati between the amount of evaporation and precipitation and temperatu the highest evaporation occurring during periods of high temperatu and little precipitation. Wind is also an important factor, and t character and duration as well as the actual amount of precipitation h a great influence upon evaporation. A long period of light rainf retards evaporation more than a short period of heavy rainfall, althou the actual amount of precipitation may be greater in the latter case.

A study of the data here presented shows that there is a gener correlation between the rate of evaporation and the rate of developme of *Aphis pomi*. On the whole, a high rate of evaporation was accor panied by a rapid development of the aphids, and a low rate of evapor tion by a comparatively slow development of the insects. While th correlation seems to be true in a general way, there is considerable vari tion from the rule. The variation which may occur in the average dai rate of evaporation during any given length of developmental period illustrated graphically in figure 7.

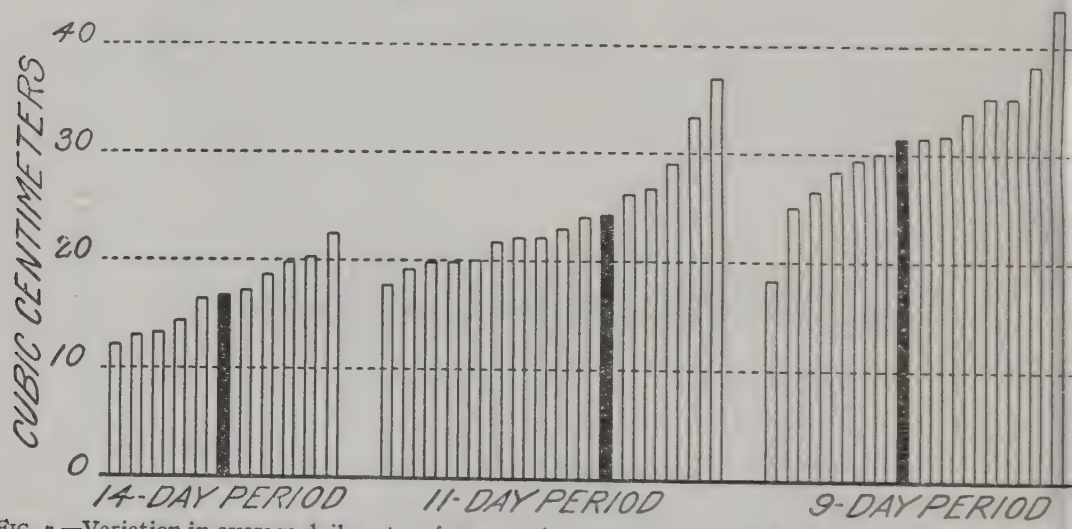

FIG. 7.—Variation in average daily rates of evaporation occurring during 14, 11, and 9 day development periods. White bars represent average daily evaporation, individual records. Black bars represe the means of all records of their respective periods.

These results show that under the conditions of this investigatio evaporation, as registered by the standard evaporimeter used, is not satisfactory measure of aphis metabolism. This condition apparentl results from the fact that the combination of factors, humidity, tempera ture, wind, etc., which influence evaporation, affect evaporation fro the standard porous cup in a manner which is not closely comparab to their effect upon the metabolism of *Aphis pomi*.

As pointed out by Livingston,[6] the rates of evaporation from differe types of evaporimeters under any given complex of atmospheric cond tions are not comparable. It is, therefore, not surprising that evapora tion from an instrument as used in these experiments would not give a accurate index of the effects of the atmospheric conditions upon aphi metabolism. It is possible that an evaporimeter more closely simulatin the conditions of the aphid body might give a closer correlation betwee atmospheric evaporation and insect metabolism.

[6] LIVINGSTON, Burton Edward. OP. CIT.

TABLE III.—*Relation of evaporation to rate of development of Aphis pomi, 1919*

Aphid series No.	Date of birth.	Date first young produced.	Developmental period.	Total evaporation.	Average daily evaporation.
			Days.	*Cc.*	*Cc.*
1	Mar. 31	Apr. 29	29	342.5	11.8
2	Apr. 29	May 17	18	410.0	22.8
3	May 17	June 2	16	294.0	18.4
4	June 2	17	15	336.2	22.4
5	3	18	15	335.5	22.4
6	4	18	14	203.6	14.5
7	5	19	14	285.9	20.4
8	6	20	14	277.8	19.8
9	9	23	14	242.7	17.2
10	10	24	14	265.9	18.9
11	12	25	13	260.7	20.0
12	13	24	11	217.4	19.8
13	17	29	12	268.8	22.4
14	18	29	11	240.2	21.9
15	20	July 1	11	243.3	22.1
16	21	2	11	253.5	23.0
17	22	3	11	265.7	24.1
18	23	3	10	248.5	24.8
19	24	5	11	288.8	26.2
20	25	7	12	296.5	24.7
21	26	8	12	304.3	25.4
22	27	8	11	295.5	26.9
23	28	9	11	320.9	29.2
24	29	8	9	256.6	28.5
25	30	10	10	316.0	31.6
26	July 1	11	10	313.0	31.3
27	2	12	10	306.1	30.6
28	3	12	9	272.6	30.3
29	4	14	10	322.4	32.2
30	6	15	9	318.0	35.3
31	7	17	10	368.4	36.8
32	8	19	11	405.5	36.9
33	9	18	9	317.9	35.3
34	11	20	9	345.9	38.4
35	12	22	10	429.0	42.9
36	13	22	9	392.5	43.6
37	14	21	7	286.1	40.9
38	16	24	8	339.4	42.4
39	29	Aug. 9	11	196.9	17.9
40	30	10	11	222.1	20.2
41	31	11	11	245.0	22.3
42	Aug. 3	11	8	192.9	24.1
43	4	17	13	382.5	29.4
44	7	17	10	309.1	30.9
45	8	18	10	305.1	30.5
46	11	19	8	238.0	29.8
47	12	20	8	254.6	31.8
48	13	20	7	238.5	34.1
49	14	23	9	304.3	33.8
50	16	24	8	237.2	29.7
51	19	28	9	286.9	31.8
52	20	30	10	320.1	32.0
53	21	30	9	285.9	31.7
54	23	Sept. 2	10	274.0	27.4
55	24	5	12	263.7	21.9
56	25	7	13	235.8	18.1
57	26	7	12	207.9	17.3
58	27	9	13	193.2	14.8
59	28	12	15	187.2	12.5
60	29	13	15	170.1	11.3

TABLE III.—*Relation of evaporation to rate of development of Aphis pomi, 1919*—Co

Aphid series No.	Date of birth.	Date first young produced.	Developmental period.	Total evaporation.	Average daily evaporation.
			Days.	*Cc.*	*Cc.*
61	Aug. 30	Sept. 22	24	392.5	16.
62	31	14	14	170.8	12.
63	Sept. 1	15	14	187.9	13.
64	2	15	13	159.0	12.
65	3	18	15	170.5	11.
66	5	19	14	183.0	13.
67	6	19	13	182.3	14.
68	8	21	13	220.8	17.
69	10	21	11	211.6	19.
70	11	23	12	319.6	26.
71	13	28	15	392.6	26.
72	16	30	14	315.7	22.

TABLE IV.—*Relation of evaporation to rate of development of Aphis pomi; summary data for 1919 and 1920*

Length of developmental period.	Average total evaporation for period.	Average daily evaporation.	Number of records.
Days.	*Cc.*	*Cc.*	
36	453.9	12.6	1
29	342.5	11.8	1
24	392.5	16.4	1
22	406.6	18.5	1
19	412.6	21.7	1
18	408.0	22.7	2
16	294.0	18.4	1
15	265.3	17.7	6
14	236.5	16.9	10
13	256.9	19.7	10
12	265.8	22.1	8
11	266.2	24.2	15
10	305.2	30.5	14
9	284.2	31.6	14
8	252.4	31.5	5
7	262.3	37.5	2

TEMPERATURE STUDIES

The data obtained during 1919 showed that, while there is a gener correlation between evaporation and the rate of metabolism of *Aphis pom* a measure of evaporation alone is not a satisfactory index to aphid deve opment. During the summer of 1920 the investigation was continued an accurate records of temperature as well as evaporation were maintaine

The daily maximum, mean, and minimum temperatures for the summe of 1920 are given in Table II and are shown graphically in figures 5 and It will be noted that there was considerable daily variation in temperatu ranging from a minimum variation of 7° F. on April 1 to the maximu variation of 54° on August 14. In general, the greatest daily variatio occurred during periods of high mean temperatures and was correlate with a high rate of evaporation. Periods of little daily variation i temperature were usually accompanied by low mean temperatures, b little evaporation, and frequently by precipitation.

Table V shows the relation of temperature to the rate of development of *phis pomi*. The actual mean temperatures of the developmental periods the several series were first plotted as shown by the circles on the aph, figure 8. It was found that these points lie approximately along

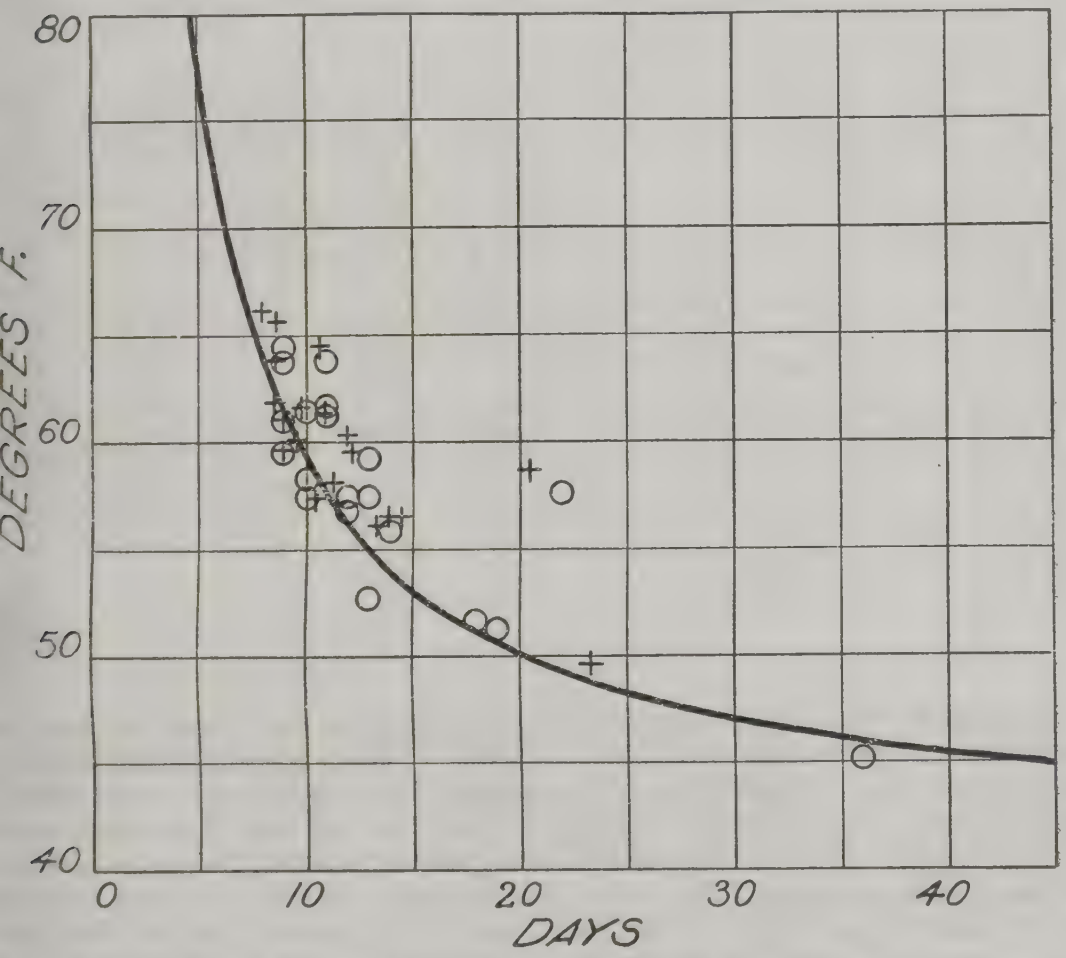

FIG. 8.—Curve showing theoretical relation of temperature to rate of development of *Aphis pomi*.

hyperbolic curve having the formula $x=\frac{a}{y-b}$.[7] This formula may be xpressed by the formula: Length of developmental period in days

$$=\frac{180}{\text{Temperature in degrees Fahrenheit}-41}.$$

If the curve thus plotted be extended it will be found that as the temerature is lowered the development of the aphids becomes less rapid, ntil at a temperature of 41° F. or less development ceases entirely. In ther words, only temperatures above 41° are "effective"[8] in the develop- ient of *Aphis pomi*. By subtracting all temperatures of 41° or less and y computing the mean of the remaining temperature readings the mean ffective temperatures (Table V) were obtained. By subtracting from ie developmental period the time during which the temperature was 41° r less the duration of effective temperature was determined. These ata were then plotted on the graph (fig. 8) as indicated by the crosses.

[7] The writer is indebted to Prof. E. B. Beaty, of the Department of Mathematics, Oregon Agricultural ollege, for the computation of the formula for this curve.

[8] SANDERSON, E. DWIGHT THE RELATION OF TEMPERATURE TO THE GROWTH OF INSECTS. *In* Jour. con. Ent., v. 3, no. 2, p. 113–139, fig. 6–26. 1910. Authors cited, p. 138–139.

TABLE V.—*Relation of temperature and evaporation to rate of development of Ap pomi, 1920*

Aphid series No.	Date of birth.	Date first young produced.	Developmental period.	Total evaporation.	Average daily evaporation.	Mean temperature.	Duration of effective temperature.	Mean effectiv temper ture.
			Days.	*Cc.*	*Cc.*	°*F.*	*Days.*	° *F.*
1.....	Mar. 28	May 3	36	453.9	12.6	45.1	23.3	49
2.....	May 3	16	13	312.4	24.0	52.6	10.4	57
3.....	13	June 1	19	412.6	21.7	51.3	14.5	56
4.....	16	3	18	406.0	22.6	51.6	13.9	56
5.....	31	12	12	249.1	20.8	56.8	11.6	57
6.....	June 2	16	14	231.5	16.5	55.9	13.3	56
7.....	9	21	12	216.8	18.1	57.5	11.4	58
8.....	14	27	13	324.0	24.9	57.5	12.2	59
9.....	17	27	10	274.7	27.5	58.2	9.5	60
10.....	21	30	9	238.1	26.5	59.5	8.6	61
11.....	27	July 6	9	265.3	29.5	64.5	8.7	65
12.....	30	9	9	302.7	33.6	63.8	8.7	63
13.....	July 7	18	11	218.8	19.9	61.1	10.9	61
14.....	9	18	9	166.7	18.5	61.0	9.0	61
15.....	18	28	10	273.6	27.4	62.5	9.8	62
16.....	28	Aug. 6	9	226.1	25.1	64.5	8.0	66
17.....	Aug. 12	23	11	368.7	33.5	63.8	10.7	64
18.....	20	Sept. 2	13	298.6	22.9	59.3	12.0	60
19.....	20	11	22	406.6	18.5	57.6	20.5	58
20.....	23	2	10	212.9	21.3	57.5	9.0	59

In general, the records as shown on figure 8 do not coincide exact with the theoretical curve of development. This is no doubt due large to the fact that observations of the aphids were made only once dail which would tend to cause a lagging in the recorded rate of developme of the insects. Toward the end of the growing season the developme of the aphids in some of the series was probably retarded to some exte by the lack of succulence of the plant tissues, in spite of the fact that t most succulent growing tips were selected for rearing the aphids. T extent to which development may be retarded by such a limiting fact is shown by series 19, which was reared upon mature foliage. For i development this series required a period of effective temperature of 20 days, although accompanied by a mean effective temperature (58.6 high enough to permit development in half the time consumed. Th effect of the growth of the plant upon the development of *Aphis pom* was also noted by Baker and Turner,[9] who regard it as a food relationshi It is evident that the condition of the foliage of the food plant frequentl constitutes a limiting factor of considerable importance to the activiti of *Aphis pomi.*

CONCLUSIONS

Under normal outdoor conditions there is a general correlation betwe atmospheric evaporation and the rate of development of *Aphis pomi.*

Atmospheric evaporation, as measured by the standard evaporimet used, does not serve as a satisfactory index to the rate of developme of *Aphis pomi.*

[9] BAKER, A. C., and TURNER, W. F. MORPHOLOGY AND BIOLOGY OF THE GREEN APPLE APHIS. Jour. Agr. Research, v. 5, no. 21, p. 983, pl. 75. 1916.

Temperature, during periods when no other factor limits the rate of development of the species, constitutes a more satisfactory index than does the rate of atmospheric evaporation.

The relation of temperature to the rate of development of *Aphis pomi* may be represented by a hyperbolic curve having the formula: length of developmental period in days $= \frac{180}{\text{Temperatures in degrees Fahrenheit} - 41}$.

Plant growth frequently constitutes a factor limiting the rate of development of *Aphis pomi* feeding on slowly growing foliage.

27976—23——5

DOWNY MILDEW ON LETTUCE IN CALIFORNIA

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INTRODUCTION

Downy mildew caused by *Bremia lactucae* Regel was reported several ecades ago by Arthur (*1*),[2] Burrill (*4*), Halsted (*6, p. 175–176*), and 'arlow (*5, p. 313*), as occurring in this country on lettuce grown under glass.)uring a long period following these early reports, investigators appear ıot to have given any intensive attention to this disease outside of Europe, where Marchal (*7*) obtained partial control of it by the applica- ion of chemicals to the soil. Recently, downy mildew was reported s causing slight losses, usually in greenhouses, in most of the North- astern States and in Ohio, Indiana, Iowa, and Texas. With the xception of these notes on the occurrence of the disease, referring o it entirely as a greenhouse problem, discussions of *B. lactucae* in litera- ure have been meager, and no attention appears to have been given o it as a pathological problem of lettuce grown in the field on a com- ıercial scale. In California, where extensive acreages are annually lanted to lettuce, downy mildew has become an important factor both ı production and transportation.

EARLY CLASSIFICATION OF THE FUNGUS

Bremia lactucae was first recorded as a parasite on lettuce and other ompositae in Europe in 1843. Although the fungus retained its osition among the Peronosporaceae, according to Saccardo (*8, p. 244*), it vas discussed later by Berkeley (*3*), de Bary (*2, p. 108*), and others under he names of *Peronospora gangliformis* (Berk.) de Bary, *Botrytis gang- ioniformis* Berk., *Peronospora ganglioniformis* Berk., *P. nivea*, *Botrytis actucae*, *Botrytis geminata*, *Botrytis sonchicola* Schlecht., and *Actinobotrys ulasnei* Hoffm.

DISTRIBUTION OF THE DISEASE IN CALIFORNIA

The most important commercial lettuce-growing areas of California re located in the Imperial Valley and the Los Angeles, Sacramento, nd Watsonville districts. During the season of 1919–20, there were pproximately 19,000 acres of lettuce in these districts, and the major ortion of the product of this acreage was destined for carlot shipments o markets in all parts of the country. The lettuce planted consists argely of the variety known as New York.[3] During the past few ears about 85 per cent of the total annual acreage has been planted to

[1] Accepted for publication July 2, 1921.
[2] Reference is made by number (italic) to "Literature cited," 993.
[3] The variety New York is also known locally as Los Angeles and Los Angeles Market. In Eastern arkets it is frequently erroneously called Iceberg. Other synonyms of this variety are Henderson's New ork, Bonanza, Schwill's Bonanza, Queen, Faust's Queen, Sterling, Hastings's Drum Head, Wonderful, nd Neapolitan.

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this variety, while the remaining 15 per cent consisted largely of t varieties known as Iceberg and Big Boston. Both New York and I berg are cabbage-heading varieties, the former wholly green and the lat greenish with a brownish tinge. Synonyms for Iceberg are Burpe Iceberg, Curled India, and Weaver's Market Gardener's. Synony were taken from the work of W. W. Tracy, jr. (*9*).

With the concentration of the industry upon one variety to the exte of 85 per cent, an increasing amount of downy mildew has develop particularly since the variety New York is highly susceptible to th disease. In the Imperial Valley the disease was found in a milder fo than in other districts. In the Los Angeles district the fungus was fou on lettuce during all months of the year but appeared to be most acti from October to May. During this period, in many fields where t plants were approaching condition for harvest, 90 per cent of the cr was affected severely, that is to say the fungus was found on most of t exposed leaves regardless of age. In a survey of the whole distr during the season of 1919–20 an estimate of 40 per cent severe infecti was made. In the Watsonville and Sacramento districts the severity the disease was similar to that in the Los Angeles district.

SYMPTOMS OF THE DISEASE

The first indications of infection appear as scattered light green yellow areas on the upper surface of the leaf. Any exposed leaf may attacked, although the oldest leaves frequently show the first signs infection. Shortly after the appearance of these discolored areas, t fungus develops on the lower surface of the leaves as patches of dow white directly beneath the discolored areas. Conidial development almost simultaneous with the appearance of the fungus on the low surface. The size of the spots varies from ½ to ¾ inch in diamet the surface development of the organism being limited by the extent spread within the tissues of the host. Frequently spots coalesce a when of long standing take on a brown color, giving rise to the te "brown margin" recently employed by inspectors of the Bureau Markets (Pl. 1).

DESTRUCTIVENESS OF THE DISEASE

The frequency and severity of infection of lettuce heads makes sel tion in the field and close trimming in packing for shipment imprac cable. In packing, the outer leaves, usually three to four in numb are removed. This amount of trimming does not reduce appreciat the amount of infection, since not only do the outer leaves contain brow lesions or spots but many of the younger leaves are covered with la infections.

For immediate local consumption lettuce so affected as to have t margins of the outer leaves browned and white patches of fungus other exposed parts is not greatly reduced in value, but when su lettuce is stored or shipped, rapid deterioration through decay is lik to occur. That a large portion of the crop is shipped out of the St is shown by the record of over 5,789 cars of lettuce shipped during t year from July, 1919, to July, 1920.

Bremia lactucae was found to remain vigorous and even to flourish lettuce in cars under refrigeration on arrival at destination. Dr. G.

K. Link, in an unpublished report, stated that the fungus was abundant on lettuce shipped from California to Houston, Tex., New Orleans, La., and Chicago, Ill. On arrival at these points, the heads were badly decayed. That such decay occurs readily was established in the laboratory, where lettuce heads, some severely affected with lesions caused by *Bremia lactucae* and some normal, were placed under similar conditions and where the affected heads always decayed more rapidly than normal heads. A number of secondary organisms in the form of species of Macrosporium, Cladosporium, Botrytis, Fusarium, and Aspergillus were isolated from lesions caused by *Bremia lactucae*, and of these several are capable of causing decay.

CAUSAL FUNGUS

Bremia lactucae has long been known as one of the Peronosporaceae. En masse, the fungus when first appearing on the outer portion of the leaf is of a snowy white color. Microscopically, both mycelium and conidia are hyaline. In the host the mycelium grows between the cell walls, projecting modified club-shaped haustoria into the cells. Aerial portions of the fungus proceed generally from stomatal openings, at which point a single issuing hypha forms a bulbous swelling. From this swelling two or three conidiophores arise, with an average length of 190 microns from the base to the first branches. The conidiophores are nonseptate and profusely branched, both dichotomously and trichotomously. At the distal end of each branch a cuplike swelling projects four or five sterigmatic and radiating branchlets, each bearing a single conidium. The conidia are ovate, hyaline, papillate, and average 18.5 by 17.5 microns in size (Pl. 2).

Reproduction takes place through direct germination of conidia by means of a germ tube, by the production of zoospores, and by formation of oospores. Heretofore, the first and third methods of reproduction have been mentioned in literature, while the second, the production of zoospores, has not been recorded up to this time. Direct germination was readily obtained when freshly collected conidia were placed in a hanging drop culture for a period of 24 hours. While the formation of germ tubes by conidia under aquatic conditions was being studied free-swimming zoospores were frequently observed. Numerous and careful preparations of cultures through selection of individual conidia led the writer to conclude that under certain conditions conidia developed zoospores, while under others they germinated directly. It was found that conidia produced during the cool months, December to March, inclusive, formed zoospores more readily than those conidia collected during the warm season of the year. The most favorable conditions for the development of zoospores were found to be darkness and a temperature in the vicinity of 10° C. After the preparation had been subjected to these conditions for a period of 24 to 48 hours, an abundance of motile ciliated spores could be observed. These spores were hyaline, globular, ciliated, motile for several hours, and about 4.2 microns in diameter. The exact period of motility was not determined. After emergence of the zoospores, the epispore collapsed quickly. The number of zoospores formed in each conidium seemed to vary; however, it was greater than eight. The media employed in this study were physiological salt solution, tap water, 0.1 per cent dextrose solution, and 0.1 per cent bacto-peptone solution. Common tap water

appeared to be as efficient as any other medium for the inducem of zoospore formation, while in dextrose solution there was a grea tendency to direct germination of the conidia. After coming to re the zoospores germinated with regularity (Pl. 2).

The complete pathological significance of zoospore production m be determined through further investigation. It is very evident th environmental conditions play an important part in the determinat between direct germination and zoospore formation. Although dir germination occurs in the absence of light and at 10° C., zoospore f mation predominates under those conditions, and one is led to conclu that zoospores provide for the reproduction of the fungus at low te peratures.

SUSCEPTIBILITY OF LETTUCE VARIETIES TO THE ORGANISM

The disease was found on lettuce during all parts of the year, b the severest infections occurred during the winter months. It w thought that the control of soil moisture during irrigation might inhi the development of the fungus. In order to accomplish such contr seed beds were constructed about 8 inches above the irrigation furr on the experiment field of the Office of Cotton, Truck, and Forage Cr Disease Investigations, located at Alhambra, Calif. Irrigation wa was then allowed to rise in the furrow to a line 4 inches below the surfa of the seed bed. This attempt to reduce soil surface moisture a subsequent condensation of moisture on the plant's leaf surfaces w futile in the winter months, because of rains and frequent fogs. Duri the rainless months a considerable amount of the fungus develop but not to as great an extent as during the rainy season.

In carrying out varietal tests of lettuce, it was soon found that t variety New York was highly susceptible to mildew, while several oth varieties showed evidence of resistance. During two consecutive yea six separate plantings were made each year. In each test 200 pla of each variety were kept under observation. In each test the contra in susceptibility between the varieties New York and Iceberg was ma edly evident. Iceberg appeared to be highly resistant. The relati susceptibility of four varieties of lettuce in two tests during the seas of 1919–20 is expressed in the following table.

Variety.	Number of plants.	Number of plants infected.	
		Test 1.	Test 2
New York	200	196	
Iceberg	200	1	
Big Boston	200	10	
Hanson	200	12	

The contrast in susceptibility between New York and Iceberg (Pl. is obviously in favor of the inferior variety. New York has supplant Iceberg in recent years on account of the production of a firmer a larger head during the winter months, the main growing season. Ic berg excels during the summer months, when high temperatures ret

the development of the New York. The latter is so well established in the trade that an advocacy of substitution by Iceberg would be viewed askance by shippers at present. On account of an unfolding of its advantages in the summer months, when a small amount of Iceberg is shipped, the two varieties rarely meet on the market in competition. The variety known as Hanson, an intermediate variety between New York and Iceberg, shows considerable resistance to downy mildew. The solution of the problem rests in the selection of individuals among the resistant varieties, for firmness and size of head.

SUMMARY

Climatic conditions in California are favorable and conducive to the growth of *Bremia lactucae* on lettuce in the field. The abundance of the fungus has given rise to an important problem in transportation as well as in the field. Reports by inspectors of the Bureau of Markets point to frequent deterioration of lettuce in transit due to this fungus, which invades the plant in the field.

The solution of the field problem has been approached in a large measure by the determination of the existence of resistance in certain varieties of lettuce.

The additional method of reproduction found in *Bremia lactucae* on lettuce in California in the form of zoospore production, heretofore unrecorded, does not change the position of the fungus in the Peronosporaceae.

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PLATE 1

Single leaf of New York variety of lettuce showing brown or old lesions caused by *Bremia lactucae*. The light areas in the left of leaf are new infections.

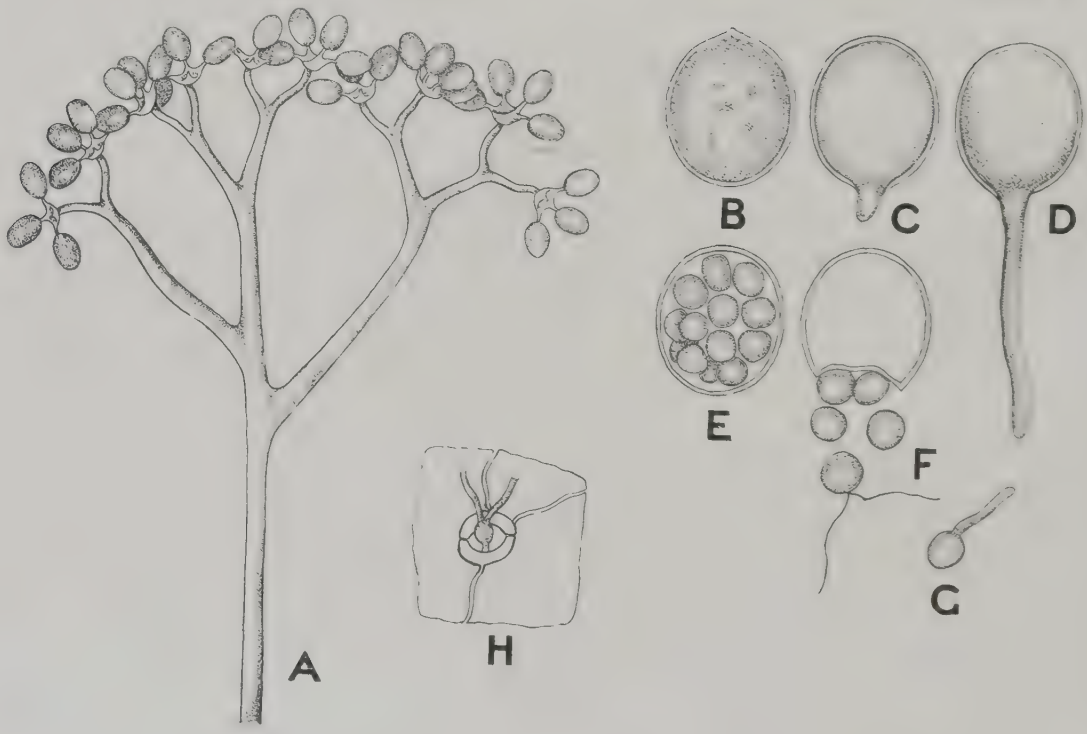
A
H
B
C
D
E
F
G

PLATE 8

Bremia lactucae Regel: Various stages in the life history of the fungus.
A.—Condiophore bearing conidia and showing arrangement.
B.—Single conidium.
C.—Germination of conidium.
D.—Conidium with zoospores ready for emergence.
E.—Zoospore after emergence from conidium.
F.—Zoospore germinating.
G.—Stoma of lettuce leaf, showing origin of conidiophore.

PLATE 3

Two varieties of lettuce from test plot, showing difference in susceptibility to *Bremia lactucae*. New York on the left shows numerous brown lesions and high susceptibility. Iceberg on the right is entirely clean; shows high resistance.

DETERMINATION OF STARCH CONTENT IN THE PRESENCE OF INTERFERING POLYSACCHARIDS[1]

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INTRODUCTION

No method of analysis by which it is possible to determine accurately the quantity of starch present in materials containing plant mucilages, pectins, or similar interfering polysaccharids has yet come to the attention of the authors. Such a method, if reasonably practicable, should be of direct aid to feed-control officials confronted with the problem of detecting adulteration in linseed meal and cake. It is an open secret that from time to time unscrupulous millers or dealers handling linseed by-products (cake, meal, and flaxseed screenings) have resorted to the following practices: The so-called "fine screenings" from flaxseed are run in with clean seed on the way to the crushers, in sufficient quantity to bring the total content of "dockage" up to about 5 per cent; flax screenings are crushed and pressed for low-grade oil, and the resulting screenings cake is ground and mixed with ground linseed cake in varying proportions; chaff, fine screenings, etc., removed from flaxseed, are pulverized and used to adulterate linseed meal.

It is impossible to determine the quantity of the adulterant, or even to prove sophistication, unless it is excessive, by making the chemical analysis usually applied to feedingstuffs (*8*),[2] because of the variations in composition of pure flaxseeds from different sources and the diverse character of the "dockage" or impurities associated with the seed. The seed of the flax plant, however, contains no starch. Therefore any starch found in a linseed cake or meal is an impurity derived from foreign or nonflax tissues present in the product examined. A small quantity of such nonflax material, however, does not necessarily constitute an adulteration. The Association of Feed Control Officials of the United States holds that the maximum quantity of weed seeds and other foreign materials legitimately permissible in linseed cake or meal is 6 per cent,[3] equivalent to 4 per cent of foreign matter in the seed before crushing. From data collected by the Bureau of Chemistry (*8*), it follows that linseed cake or meal containing less than 38 per cent of crude protein, more than 11 per cent of crude fiber, or more than 2 per cent of starch, when calculated to a moisture-free and ether-extract-free basis, is open to the suspicion of having been adulterated. As the starch content of the nonflax material normally associated with flaxseed would rarely, if ever, be greater than 50 per cent,[4] any linseed cake or meal containing more than 3 per cent of starch should be considered to be adulterated.

[1] Accepted for publication Feb. 20, 1923.

[2] Reference is made by number (italic) to "Literature Cited," p. 1006.

[3] In the definitions of the association there is the further provision that no portion of the stated 6 per cent of weed seeds and other foreign materials shall be deliberately added.

[4] This opinion is based on the nature of the flaxseed "dockage" examined for a large number of samples of country, terminal, and mill seed (*8*).

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An accurate method for the determination of starch in linseed by-products could be used also to determine starch in apple pomace and other pectin-bearing materials. It would doubtless be of value also to analysts engaged in researches in dietetics, in determining the starch content of diabetic foods containing troublesome plant slimes and mucilages, for example.

There are two serious difficulties in the determination of starch in material like linseed meal: (a) It is nearly impossible to effect the preliminary elimination of sugars [5] and other interfering carbohydrates by extraction with cold water or 10 per cent alcohol, as required in the official methods (*1, p. 95–96*), because the mucilaginous substances present in linseed meal form an almost impervious layer on the filter, greatly retarding percolation, if not preventing it. (b) Linseed mucilage, like other vegetable mucilages and pectins, is a polysaccharid, and must be excluded from the material subjected to acid hydrolysis. Otherwise it will yield reducing sugars, thereby increasing the apparent starch content.

The official direct acid hydrolysis method is therefore barred from use at the outset. The official malt-diastase method, based on selective carbohydrate hydrolysis,[6] can not be employed because of the first difficulty mentioned. Even if the problem of washing out interfering sugars be solved, after the malt digestion the mucilage has lost none of its capacity for obstructing filtration and it is practically impossible to separate the starch conversion products from the rest of the material in aqueous solution.

There are, of course, colorimetric methods, in which advantage is taken of the blue color of the compounds of starch with iodin. One of the most promising modifications of this type, that of Cassal (*2*), was employed in estimating the starch content of apple pomace. Because of differences in the quality of color obtained and consequent difficulty in establishing an accurate comparison between the depth of this color and that of the starch-iodin standard, however, it was thought that this type of method could not meet the requirements in accuracy and dependability.

EXPERIMENTAL

PRELIMINARY METHOD

Because of its coagulating effect on colloidal polysaccharids, such as pectins, mucilages, etc., strong alcohol can be used to eliminate these substances by precipitation, after conversion of the starch by selective enzyms. For this to be of value, however, the starch conversion products must remain in solution in the same strong alcohol.

The work of Davis and Daish (*3*) indicated that by employing taka-diastase as the amylolytic enzym this condition could be fulfilled. These investigators reported that by digesting with taka-diastase at 38°C. for a sufficient length of time, starch is quantitatively converted into maltose and dextrose, and into these sugars only. Neville (*5*) has shown that this enzym has no effect on linseed mucilage, and Schneider (*6*) reported that it does not hydrolyze apple pectin.

[5] Linseed meal normally contains from 3 to 4 per cent of nonreducing sugars, calculated as sucrose. A trace of reducing sugars is usually present. Similar results have been reported by Van Kampen (*7*).

[6] The mucilage of linseed meal is not hydrolyzed by the diastase of barley malt, according to Neville (*5*) nor is apple marc and its pectin, according to Schneider (*6*).

Accordingly, the starch content of several linseed meals, the approximate degree of purity of which had been ascertained,[7] (*8*) was determined by a method of which the following are the salient points:

After extracting with ether, the charge was leached with 70 per cent alcohol [8] to remove part at least of the sugars and other interfering substances. To correct further for any effect that might be exerted by interfering substances not extracted by the 70 per cent alcohol, a control determination was conducted, in which the starch hydrolysis was omitted and in which only the effect of the soluble carbohydrates was measured.

Hydrolysis of the starch was effected by digestion with taka-diastase, following the method of Davis and Daish (*3*).

Mucilage and other interfering polysaccharide were coagulated by 75 per cent alcohol and separated from the soluble starch conversion products, presumably maltose and dextrose, by filtration. After eliminating alcohol from the filtrate by evaporating over steam, the starch conversion products were hydrolyzed by acid and the dextrose was determined. The results obtained were corrected by deducting first the dextrose in the taka-diastase blank, and, second, the dextrose representing the control determination in which the starch was not hydrolyzed.

RESULTS OBTAINED WITH PRELIMINARY METHOD

The results obtained by this method on the linseed cakes and meals examined and the proximate analyses of these products are given in Table I.

TABLE I.—*Starch in linseed by-products of known composition* [a]

Material analyzed.	Composition on original basis.							Composition on moisture-free and ether-extract-free basis.				
	Nonflax matter.	Moisture.	Starch.	Ether extract.	Crude protein.	Crude fiber.	Ash.	Starch.	Crude protein.	Crude fiber.	Ash.	Starch content of nonflax matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Adulterated linseed meal (Sample 26291)	[b]28.0	10.0	7.2	6.8	30.5	10.0	5.8	8.7	36.6	12.0	7.0	25.7
Linseed cake containing excessive foreign matter (traced) (Sample 27243)	8.6	9.6	1.4	5.9	34.8	8.1	5.6	1.7	41.1	9.6	6.7	16.2
Linseed cake (traced) (Sample 27247)	3.8	10.2	1.4	6.8	34.8	8.1	5.3	1.7	41.9	9.7	6.4	36.8
Linseed cake (traced) (Sample 27262)	3.3	10.5	[c]1.0	6.7	35.8	7.8	5.1	1.3	43.2	9.4	6.1	[c]40.9
Adulterated linseed meal (Sample 27274)	[b]14.0	9.7	1.4	7.2	33.8	8.6	5.6	1.7	40.6	10.4	6.8	10.0
Flax screenings cake (traced) (Sample 27275)	89.8	9.4	16.3	8.4	17.9	16.0	8.3	19.8	21.8	19.5	10.1	18.2
Nonflax matter from flax screenings (composition calculated)	100.0	.....	.....	.....	.....	.....	.....	22.0	19.1	23.5	9.4	.....
Linseed cake (traced) (Sample 27276)	5.6	10.5	1.8	5.0	34.9	8.1	6.5	2.1	41.4	9.5	7.7	32.1

[7] These meals were prepared from traced linseed cakes. The investigator took samples from the seed entering the rolls, traced the seed through the crushing, cooking, and pressing, and took a representative section of the resulting cake. The percentage of nonflax material in the seed was ascertained without difficulty. As practically all of the expressed oil comes from the flaxseed and for practical purposes constitutes one third of the weight of the seed, it follows that the percentage of foreign matter in the cake or meal is 1½ times that in the seed crushed.

[8] It was subsequently shown that 35 per cent alcohol percolates through linseed meal with almost equal facility and is, of course, a better solvent for interfering carbohydrates (p. 998).

[a] The starch was determined by the taka-diastase method, corrected by a "control" determination.

[b] The content of nonflax material in this sample was calculated from data obtained through inspection of the mill.

[c] From subsequent work, the true starch content is believed to be 1.34 per cent (1.62 per cent on the dry fat-free basis).

This method was tedious and open to the objection that nothing definite was known as to the fate of the interfering carbohydrates, other than the mucilage, during the taka-diastase digestion. Furthermore, Horton (*4*) questioned the validity of the conclusion of Davis and Daish, that starch was quantitatively converted by taka-diastase into maltose and dextrose.

REVISED METHOD

The apparent solubility in rather strong alcohol of the conversion products obtained through a properly conducted malt-diastase digestion of starch was considered.[9] Experiments were undertaken to test the feasibility of using strong alcohol as the medium for separating such starch conversion products from mucilage or other interfering p lysaccharids. Neither the dextrine nor any other conversion product obtained by digesting starch with an infusion of barley malt (under conditions fully described on p. 1003–1004) was thrown out of solution [10] by alcohol of 60 per cent strength. Starch determinations made on 0.8 and 1.2 gm. charges of prepared starch, in which the conversion products were subjected to the action of 60 per cent alcohol, yielded results accounting for 100 per cent of the starch known to be present. On the other hand, alcohol of this strength served perfectly to coagulate and precipitate linseed mucilage and apple pectin. It was shown also that 35 per cent alcohol served well in the preliminary extraction of sugars and other carbohydrates that affect the results in a determination of starch.

A new method of analysis, consisting of the following steps, was devised: (a) Preliminary extraction with 35 per cent alcohol;[11] (b) conversion of the starch by an infusion of barley malt; (c) separation of mucilage or other interfering polysaccharid by its coagulation and precipitation in 60 per cent alcohol; (d) elimination of alcohol from the filtrate by evaporation and acid hydrolysis of the starch conversion products to dextrose; (e) defecation of the acid dextrose solution by phosphotungstic acid; and (f) determination of the dextrose by copper reduction.

RESULTS OBTAINED WITH REVISED METHOD

In order to test the accuracy of the method, determinations were made on charges containing known quantities of added starch. The starch used for this purpose was prepared by washing a good grade of cornstarch with water until the extract gave no blue color with iodin, washing with 95 per cent alcohol and then with ether, and finally drying in a vacuum oven at 65° to 70° C.

Analysis of this prepared starch gave the following results: Starch by the official acid hydrolysis method,[12] average of 4 determinations, 87.62 per cent; starch by the malt-diastase method, with the 60 per cent alcohol purification, average of 2 determinations, 87.19 per cent; ash, 0.10 per cent; nitrogen by a micro-kjeldahl determination, 0.077 per

[9] H. C. Gore, of the Bureau of Chemistry, showed that a heavy malt sirup formed a clear solution in alcohol of a concentration of more than 60 per cent.

[10] The highest concentration of starch conversion products tested was that corresponding to 1.5 gm. of original starch to 500 cc. of the 60 per cent alcohol. No turbidity developed overnight. In 80 per cent alcohol some turbidity was noticeable.

[11] Twenty-five per cent alcohol, being a better solvent, serves best with materials that are not impervious to it.

[12] This check determination was made by J. I. Palmore, of the Food Control Laboratory, Bureau of Chemistry.

cent. A combustion determination[13] of carbon and hydrogen gave the following results: Carbon, 40.67 per cent, and hydrogen, 6.68 per cent, indicating a hexosan content of 91.53[14] per cent and a water content (uncombined) of 8.79 per cent.

The foregoing determinations were made on the starch near the close of the experimental work, several months after the samples had been prepared. The material was quite hygroscopic, and the percentage of starch determined by modifications of the official malt-diastase method was higher at the time of preparing the starch than four and five months later. The percentage of starch ranged from 89.65 to 87.19, owing, it is believed, to the absorption of moisture from the air. The values accepted as representing the true starch content of this standard sample are 88.9 per cent for the earlier experiments and 87.5 per cent for the later work. Throughout this study, the factor 0.90 was used in calculating starch from dextrose. The factor 0.93 probably more nearly represents the actual yield (*1*, *p.* 95). Had this factor been used, the accepted values would have been 91.9 per cent for the earlier and 90.4 per cent starch for the later work. The validity of the figures showing the value of the methods of analysis is not affected by the factor used.

Working with dried apple by-products, with and without added starch, the new method was compared with the Cassal colorimetric method and the official malt-diastase method. The results obtained were not entirely satisfactory. While the official method yielded results that were too high, owing undoubtedly to the inclusion of some pectin, the alcohol precipitation method failed to fully account for all the starch present. The results obtained on a sample of linseed meal containing known quantities of starch also were unsatisfactory in this first test with the method.

CORRECTION OF REVISED METHOD

It was thought that the failure to account for all starch known to be present might be due to: (a) Formation of mucilaginous lumps in the material, during gelatinization, which subsequently served to shield particles of starch from the dissolving action of the malt extract; (b) failure to thoroughly break up the ropy coagulum formed in the 60 per cent alcohol, with consequent occlusion of some of the more concentrated portions of the solution; (c) adsorption of maltose and dextrine by the coagulated colloids.

Accordingly, the method of analysis was reviewed to correct any errors in technic. The details and precautions to be observed are fully set forth in the final statement of the method of analysis advocated (p. 1002–1005).

If adsorption of the starch conversion products were primarily responsible for the low results, it seemed reasonable to expect that this error could be obviated by double precipitation with the 60 per cent alcohol.

TESTING FOR ADSORPTION BY DOUBLE PRECIPITATION

After the first precipitation in 60 per cent alcohol, the 500 cc. of liquid was thoroughly mixed with the coagulum, by pouring the mixture

[13] The determinations of nitrogen, carbon, and hydrogen were conducted by J. F. Ellis, of the Nitrogen Section, Bureau of Chemistry.

[14] A small quantity of cell wall tissue (cellulose) in the sample counts as starch in a combustion determination.

back and forth from one container to another, and filtered through dry paper, care being taken to avoid the loss of any of the solution or material.[15] The filter was allowed to drain until there was no danger of loss of filtrate on removing the paper and its contents from the funnel. The total volume of filtrate that had drained through was ascertained. The aliquot represented by it[16] was designated F and the whole solution, Solution 1. Using about 150 cc. of hot water, the filter paper and coagulum contained in it were returned to the 500 cc. volumetric flask, care being taken to rinse back all material adhering to the containers previously used in the mixing operation. (This serves to redissolve the mucilage, and the solution also contains that portion of Solution 1 which did not drain through the filter.) The aqueous mixture in the 500 cc. flask was shaken hard and thoroughly and, after cooling, the colloids were reprecipitated with 316 cc. of 95 per cent alcohol, the precaution of adding the alcohol in small portions and mixing to keep the coagulated material broken up being taken.

The total volume of liquid was completed to 500 cc. with water, mixed as before, by pouring it back and forth, and filtered through dry paper. This filtrate is called Solution 2. Aliquots of 200 cc. of Solution 1 and Solution 2 were evaporated in separate beakers, over steam, and, after the usual acid hydrolysis and purification, the dextrose in each aliquot was determined.

The additional determination of dextrose can not be avoided when double precipitation is employed. Hypothetically, the charge of starch rendered soluble by the diastase digestion, after the precipitation of colloids by alcohol, may be considered as existing in two forms: Unadsorbed portion (S), freely in solution in the 60 per cent alcohol; and a portion (A) supposedly adsorbed by the coagulum.

As S is entirely in solution, its quantity is readily obtainable from the quantity of dextrose found in the aliquot of Solution 1. The estimation of A, however, is not so simple. In the second alcohol extract (Solution 2) there is presumably not only all of A[17], but also $\frac{500-F}{500}$ of S. (The quantity $\frac{500-F}{500}$ represents the aliquot of Solution 1 that adhered to the filter and coagulum.) Thus the dextrose determined on the 200 cc. aliquot of Solution 2 is represented by $\frac{200}{500}(A+\frac{500-F}{500}\cdot S)$ and the dextrose determined on the 200 cc. aliquot of Solution 1 is represented by $\frac{200}{500}\cdot S$. In the two equations indicated, F is known, and S and A are readily obtainable. The total dextrose representing the starch in the original charge should equal $S+A$. This value, of course, must be corrected for the dextrose determined in the malt blank. This correction properly is made by calculating back to the quantity of dextrose in the malt infusion added to the mash, and subtracting this directly from $S+A$. The remainder, multiplied by 0.9, gives the starch content.

[15] A correction was obtained for dextrose due to the malt infusion and filter paper, by proceeding with the malt blank determination in exactly the same way.

[16] The aliquot may be accurately and conveniently determined by weighing the original 500 cc. of solution (by deducting the weight of solid material from the total), and also the filtrate, on a balance sensitive to 1 gm.

[17] Theoretically this would not be strictly true, owing to the proportional distribution of solute maintained between solution and adsorbent. The quantity of starch conversion products so withheld from solution however, would be negligible, because of the dilution.

RESULTS OBTAINED WITH CORRECTED REVISED METHOD

Starch determinations by the new method, with the added feature of double alcohol precipitation, were made on a sample of linseed meal of known degree of purity and on mixtures of this linseed meal and known quantitites of starch. This linseed meal (Sample 27262) was a ground traced cake, and, as calculated from the foreign matter in the seed before crushing, contained 3.3 per cent of nonflax material. Analysis showed it to have the following percentage composition: Moisture, 10.5; ether extract, 6.7; crude fiber, 7.8; crude protein, 35.8; ash, 5.1.

The results obtained by a single 60 per cent alcohol defecation (represented by *S*), as well as by the double precipitation on the same charges, are interpreted in Table II.

TABLE II.—*Comparison of the single and double alcohol precipitation modifications of the malt-diastase method for starch in linseed meal*

Material analyzed.	Weight of linseed meal.	Weight of prepared starch added.	Total starch present (pure starch).	Total dextrose after 1 alcohol precipitation from Solution 1 (*S*).	"Adsorbed" dextrose from Solution 2, after subtracting $\left(\frac{500-F}{500}\cdot S\right)$, (*A*).	Total dextrose after 2 alcohol precipitations (*S*+*A*).	Starch by single alcohol precipitation method.				Starch by double alcohol precipitation method.			
							Weight of starch after correcting for malt control; derived from *S*.	Total.	Average.	Divided by total starch present.	Weight of starch found after correcting for malt control; derived from *S*+*A*.	Total.	Average.	Divided by total starch present.
	Gm.	*Gm.*	*P.ct.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*	*P.ct.*	*P.ct.*	*Gm.*	*P.ct.*	*P.ct.*	*P.ct.*
Control (dextrose correction on malt infusion, etc.)				0.35625	0.02875	0.3850								
				.35937	.04023	.3996								
Prepared starch (sample 40125)		1.5	87.5	1.82344	.01003	1.8335	1.3190	87.94	87.19	99.6	1.2971	86.47	86.65	99.0
				1.79844	.04101	1.8394	1.2965	86.44			1.3024	86.83		
Linseed meal (sample 27262)	4.0		1.34	.42031	.03033	.4506	.0563	1.41	1.34	100.0	.0525	1.31	1.30	97.0
				.41406	.03553	.4496	.0507	1.27			.0516	1.29		
Linseed meal and starch	3.8	0.2	5.65	.60312	.03056	.6337	.2208	5.52	5.49	97.2	.2173	5.43	5.27	93.3
				.60000	.01883	.6188	.2180	5.45			.2039	5.10		
Do	3.5	.5	12.11	.89531	.00880	.9041	.4837	12.09	11.87	98.0	.4606	11.52	11.35	93.7
				.87500	.01423	.8892	.4655	11.64			.4472	11.18		
Do	3.0	1.0	28.88	1.37344	.00797	1.3814	.9140	22.85	22.85	99.9	.8902	22.25	22.23	97.2
				1.37344	.00600	1.3794	.9140	22.85			.8884	22.21		

The second alcohol precipitation was not only unnecessary, but it became a source of error because of the increase in the value of the malt control. The dextrose determined on Solution 2 (*A*) does not represent adsorbed material. At least if there was any adsorption of starch conversion products, it was so slight as to be practically negligible.

Other conditions being constant, the weight of the solute [18] adsorbed should be proportional to the weight [19] of the adsorbent (in this case the coagulum). The quantity of coagulum being constant, the weight of solute adsorbed should increase with the concentration of the solute.

[18] By solute in this case is meant starch conversion products (maltose and dextrine).

[19] The adsorption is proportional to the active surface area of the adsorbent. This is assumed to be proportional to the weight.

In Table II, *A* is greatest for the malt controls, in which the concentration of solute was lowest and the quantity of coagulum was almost negligible.[20] Taking the other extreme (the mixture containing the largest quantity of added starch), the values for *A* are the smallest reported, while the concentration of solute was the second highest and the quantity of coagulum was relatively large.

While the indications are that there is little adsorption, if any, an attempt to explain from the data at hand the high relative values for *A*, particularly for the malt controls, must consist largely of theorizing. The quantities of cuprous oxid actually obtained (from an aliquot of Solution 2), on which the values for *A* are based, however, were small, ranging from 8.8 to 23.6 mgm. The application of any substantial correction for the effect on the copper reduction of the reagents used in defecation [21] to these results would greatly reduce the values for *A*, and the effect would be emphasized in the case of the malt controls, for which the yield of cuprous oxid from Solution 2 was smallest.

The results obtained by carefully following this method, using a single precipitation with 60 per cent alcohol, furnish sufficient evidence of its fundamental soundness, and the investigators recommend it for the determination of starch in materials which, like linseed meal and apple pomace, contain interfering polysaccharids. In using the method, however, the analyst must give painstaking attention to details, particularly to those operations dealing with the colloidal substances, such as the gelatinizing to a smooth paste, the thorough breaking up of coagula, and the subsequent mixing. It is also highly important to control carefully the conditions that prevail during the dissolving and conversion of the starch. Work conducted in the Bureau of Chemistry has shown that the starch is best brought into solution by starting the first digestion with malt infusion at a temperature well below 55° C., then slowly raising it to 70°, holding it there for the specified time, and continuing to increase the temperature to 80°. While the saccharifying enzyms (the maltase) are believed to be destroyed by temperatures of 70° and higher, other starch liquefying enzyms present in the malt infusion are more active at the higher temperatures, and complete solution of the starch is attained. The second saccharifying digestion is conducted at 55°.

Because of the difficulties mentioned, the method of analysis is discussed in greater detail than would ordinarily be necessary.

METHOD RECOMMENDED

PREPARATION OF MALT EXTRACT

Use clean, new barley malt of known efficacy and grind it only as needed. Grind the malt well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt, just before it is to be used. For every 80 cc. of the malt extract required digest 5 gm. of the ground malt with 100 cc. of distilled water, at room temperature, for 2 hours, or for 1 hour if the mixture can be stirred by

[20] The nature of the small quantity of coagulum from the malt controls differed from that obtained from linseed meal. This might have significance had there been sufficient adsorption to be of importance.

[21] There is some cause for believing that the use of phosphotungstic acid slightly increases the reduction of copper in Fehling's solution.

an electric mixer for periods of 5 minutes three or four times. Filter to obtain a clear extract. (It may be necessary to return the first portions of the filtrate to the filter.) Mix the infusion well.

PREPARATION OF CHARGE

Weigh out a definite charge of from 2 to 6 gm.[22] of the finely pulverized and well-mixed sample,[23] using the smaller charges in the case of materials containing much gel-forming substance. (The weight of starch in the charge must not exceed 1.5 gm.) Transfer to a dry filter paper held in place[24] in a glass funnel of the usual type. It is not necessary to use a hardened filter; any tight, high-grade paper, 12½ or 15 cm. in diameter, will be satisfactory.

EXTRACTION OF CHARGE

Extract the charge with 5 successive portions of ethyl ether, taking for each portion more than enough to cover the charge. Use a cover glass to retard evaporation. After completing the extraction, allow the ether to evaporate and extract the charge with weak alcohol. The concentration of the alcohol may be varied somewhat to suit the material under examination. For linseed meal 35 per cent alcohol (by volume) must be used, while for dried apple pomace 25 per cent alcohol is best. Use 300 cc. of the alcohol to obtain the required thoroughness of extraction. Follow this with several filterfuls of 95 per cent alcohol, and finish the leaching operations with a second ether extraction. (It is convenient to have the charge stand overnight at this point to allow the ether and alcohol to evaporate, as alcohol must be eliminated before starting the digestion with malt.)

Start the preparation of the malt infusion.

A correction for the dextrose in the malt extract is obtained by conducting a control determination, preferably in duplicate. Starting with a piece of the filter paper extracted with alcohol, distilled water is added, and the control is carried along side by side with the actual starch determination, being subjected to the gelatinization temperature, receiving the same quantities of malt extract, and being treated similarly in every respect.

GELATINIZATION

To return to the primary determination, transfer the paper and charge (free from more than traces of alcohol) to a 300 cc. Erlenmeyer flask, and mix well with from 20 to 30 cc. of distilled water, macerating paper and material to give a perfectly smooth paste. Add 100 to 125 cc. of boiling water. Mix quickly, but thoroughly, and with constant stirring. Then heat the contents of the flask until it boils freely. In the case of mucilaginous materials like linseed meal it is necessary to transfer the flask to a boiling water bath to complete the gelatinization. Gelatinize thoroughly, without scorching or adhesion of the material to the bottom of the flask. The mixture should be smooth and free from lumps.

[22] Charges of 4 gm. for linseed meal, or 3 gm. for dried apple pomace, have been found to be satisfactory.

[23] The entire sample should be ground to pass freely through a sieve of not less than 40 mesh to the inch. It is preferable to have the material sufficiently fine to freely pass a 60-mesh sieve.

[24] An ordinary paper clip serves well to clamp the paper in place.

MALT-DIASTASE DIGESTION

Cool to 50°C. or lower, add 20 cc. of the malt infusion, to controls as well as to charges, and place the flasks in a temperature-controlled water bath. Keeping the mash thoroughly mixed, gradually raise the temperature to 70°, taking from 20 to 30 minutes to accomplish this. Maintain at 70° for 30 minutes, stirring the mixture from time to time. Then increase the temperature to 80° and hold it there for 10 minutes. Finally heat to the boiling point. Keep the mixtures well stirred, as this amounts to a second gelatinization.

Cool the contents of the flasks and the water bath to 55° C. Add 20 cc. of the malt extract, mix well, and hold at 55° for 1 hour, stirring about once every 10 minutes. At the termination of the digestion rapidly increase the temperature to above 80°.

DEFECATION WITH 60 PER CENT ALCOHOL

The total volume of the hot mash should not exceed 200 cc. Transfer each mash to a 500 cc. volumetric flask. A little hot water may be used for rinsing, provided the total volume of the mixture does not exceed 200 cc. Reserve the flask for subsequent rinsing. Measure out 316 cc. of 95 per cent alcohol. Add a portion, a little at a time, to the contents of the flask, with thorough shaking between additions. As soon as enough alcohol has been added to coagulate the colloidal matter, allow the coagulum to settle somewhat, and pour a little of the supernatant liquid back into the Erlenmeyer flask used in the digestion, thoroughly rinsing the contents into the volumetric flask. Complete the addition of the 316 cc. of strong alcohol, with constant mixing, avoiding any loss of material, and, after cooling to room temperature, adjust the volume with water so that the quantity of liquid is 500 cc. (Allowance is made for the volume occupied by the charge by adding 3 cc. of water for every 4 gm. of charge present, after bringing the contents of the flask up to the 500 cc. mark.)

The starch conversion products from the original charge should be contained in the 500 cc. of 60 per cent alcohol. The determination may be interrupted at this stage for several days, if need be, but it would be necessary to readjust the volume of solution if there were a change in temperature.

Mix thoroughly, breaking up any ropy coagulum as much as possible, by pouring back and forth from one large breaker to another. Filter through dry paper. Test the solid residue for starch, either microscopically or by the iodin color test, after elimination of alcohol and gelatinization with water. If more than the merest trace of starch is found, reject the entire determination. Evaporate exactly 200 cc. of the filtrate on a steam bath to a volume of from 15 to 20 cc., or until practically all alcohol has been expelled. Do not allow the evaporation to proceed to dryness.

ACID HYDROLYSIS

Transfer the aqueous residue of starch conversion products to a 200 cc. volumetric flask with hot water, using a rubber policeman to recover all of the dextrine. Allow to cool somewhat, and complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc. of hydrochloric acid (sp. gr. 1.125), and connect the flask with a reflux condenser. Heat in a boiling water bath for 2½ hours.

PURIFICATION OF DEXTROSE SOLUTION AND DETERMINATION OF DEXTROSE BY COPPER REDUCTION

Cool and, in the case of linseed meal or other material yielding solutions which at this stage need further purification, add not more than 1 cc. of a 10 per cent solution of phosphotungstic acid in 1 per cent hydrochloric acid. Mix and allow to stand for 15 minutes at least. Increase the volume with water to 250 cc. in a volumetric flask, mix well, and filter through dry paper. Partially neutralize 200 cc. of the filtrate while stirring by adding 10 cc. of a heavy solution of caustic soda (44 gm. of sodium hydroxid per 100 cc. of the cooled solution) and nearly complete the neutralization with a little powdered sodium carbonate.[25] Transfer to a 250 cc. flask with water, cool to room temperature, make up to the mark, and mix well. Filter, if necessary, and determine the dextrose in a 50 cc. aliquot of the filtrate, by copper reduction, employing the gravimetric method either of Munson and Walker or of Allihn. Correct the weight of dextrose obtained by the weight of dextrose [26] found for the same aliquot of the malt control, and multiply the corrected weight of dextrose by 0.90 to obtain the weight of starch. (This factor 0.90 represents the theoretical ratio between starch and dextrose and was used throughout this study, although several other investigators (*1*, *p. 95*) have shown that the factor 0.93 more nearly represents the actual yield.)

ALIQUOTS

$$\text{Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250},$$

or,

$$\text{Charge} \times 0.064.$$

SUMMARY

A method for the accurate determination of the starch content of material containing interfering polysaccharids is needed as an aid in the examination of impure linseed meals, apple products, and other substances containing important quantities of mucilage, pectin, or carbohydrates of that character.

Linseed meal is sometimes adulterated with weed seeds and other impurities. Unless these impurities are present in excessive quantities it is impossible to demonstrate such sophistication by the usual chemical analysis. The pure seed of the flax plant, however, contains no starch. As the nonflax material normally associated with flaxseed would contain no more than 50 per cent of starch, and the allowable limit of such nonflax material is 6 per cent,[27] any linseed meal containing more than 3 per cent of starch may be held to be adulterated.

The interfering polysaccharids are a serious obstacle to a correct determination of starch. Plant mucilages when moistened are practically impervious to water or weak alcohol. This prevents the leaching out of sugars by these solvents. Unless the mucilages and pectins, which are polysaccharids, are eliminated before the acid hydrolysis,

[25] Anhydrous carbonate is preferable, as it dissolves rapidly.

[26] In the official A. O. A. C. diastase method (*1*) the direction to "correct the weight of reduced copper" by that found in the malt blank is wrong.

[27] None of this 6 per cent shall be deliberately added.

they yield reducing sugars, thereby increasing the apparent starch content.

Several methods of analysis were formulated and tried, with varying degrees of success. The method finally adopted is based on: (a) Extraction of the material with ether, alcohol of from 25 to 35 per cent strength, depending on the material, alcohol of full strength, and finally again with ether to eliminate interfering soluble substances; (b) gelatinization and conversion of the starch by digestion with an infusion of barley malt; (c) elimination of interfering polysaccharids by precipitating these substances in 60 per cent alcohol; (d) evaporation of the filtrate to drive off the alcohol, and subsequent acid hydrolysis of the starch conversion products; (e) defecation of the resulting dextrose solution with phosphotungstic acid; and (f) determination of the dextrose by copper reduction.

A sample of starch for use as a standard was prepared, and definite quantities were added to charges of linseed meal of known purity. These charges were analyzed by the proposed method. From 97.2 to 99.9 per cent of the starch known to be present was accounted for by the determinations.

The possibility of adsorption of the starch conversion products by colloids coagulated by the 60 per cent alcohol was investigated, by precipitating twice with 60 per cent alcohol and analyzing both filtrates. If there is any adsorption, for practical purposes the amount is negligible.

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BIOLOGICAL NOTES ON THE HEN FLEA, ECHIDNOPHAGA GALLINACEA[1]

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With the considerable increase in poultry production in the Southwest the hen flea (*Echidnophaga gallinacea* Westwood) is becoming more and more of economic importance. For this reason a biological study of the flea has been made at Uvalde, Tex., as time has permitted and as opportunity to obtain material has been presented. The work has consisted of observations on life history, seasonal abundance, and natural control, and some investigations on artificial control.

METHODS USED IN OBTAINING THE LIFE HISTORY

It was found very difficult to breed the fleas under laboratory conditions that would permit observation of individual development. The immature stages require an almost ideal condition for development. The degree of moisture in the breeding media must have a very narrow range of fluctuation, and the range of temperature within which the fleas will develop is probably rather narrow, although this point has not been very definitely determined. Apparently the optimum temperature ranges from 70° to 80° F.

For individuals or only a few specimens 5-gm. shell vials were used. Two cm. of fine dust screened from the floor of a chicken coop in which chickens heavily infested with the adult fleas were kept were placed in the tubes, care being used to obtain as much of the flea excreta as possible. This was collected by placing a heavy paper in the coop at night and removing it about sunrise the next morning The débris was then screened, and the screenings were examined for eggs. The tubes were closed by using a piece of sponge dipped in water, squeezed nearly dry, and stuffed loosely into the mouth of the tube. Pint Mason jars and tumblers holding about a half pint were used and filled about half full of the screenings. A moistened sponge was pinned to a muslin cover and suspended in the jar. It was unnecessary to use the sponge in larger containers unless the humidity was very low, but it was necessary to cover the container with a muslin cover rather than a close-fitting cover such as the glass top to the museum jars used. These jars were about 5 inches in diameter and held about 3 quarts.

INCUBATION

The incubation period has been found to vary from a minimum of 4 days to a maximum of 14 days, although the usual period is from 6 to 8 days. The minimum period was obtained when the temperature was

[1] Accepted for publication June 12, 1922.

quite uniform, with a maximum of 86° F., a minimum of 69° and an average of 76°. The maximum period was obtained in a mass in a tumbler in which the incubation varied from 9 to 14 days. Records of the temperature of the room in which the jars were kept were not obtained, but the temperature was held rather uniformly at about 65° during the day. The lowest temperature observed in the room was 43°. The average temperature in the insectary was 47°, the maximum 80°, and the minimum 17°. The low temperature was registered on the second day, and several of the eggs failed to incubate; these were probably the ones on the outer edge of the mass. About 43° may be considered a fatal temperature for the eggs.

LARVAL DEVELOPMENT

The larvæ usually begin to feed on the adult excreta within a few minutes after emergence and develop rapidly for the first few hours. The excreta of the adult are apparently necessary for the development of the young, and these latter have never been observed to feed on any other material. The fatal minimum temperature is about 50° F., and all the larvæ in an incubator died within a few hours when the temperature was 100° near the tube in which they were placed. The minimum larval period was 14 days; the maximum, 31 days.

PUPAL PERIOD

The mature larvæ construct cocoons of silk and dust. Apparently they prefer to fasten them to some firm object, as a large percentage made the cocoons against the side and bottom of the breeding container and in many cases the pupæ could be observed through the glass. The pupal period was found to be from 9 to 19 days.

ADULT DEVELOPMENT AND HABITS

The adults were inactive during the first few days after emergence and usually did not attach to a host until the fifth or eighth day. The females became engorged and oviposition began in from 6 to 10 days after attachment. Females deposited from one to four eggs per day, and oviposition took place during both day and night.

The following notes were made on the act of oviposition: A well engorged flea was observed in the act of passing excreta and in 18 minutes it was observed to be passing an egg. In 45 seconds the egg was thrown with considerable force and was apparently free for several seconds before it was thrown. While examining a flea on the head of a chicken a bright drop of fluid appeared; in 10 seconds an egg appeared, and in less than 5 seconds it was thrown with considerable force. The adult was observed to pass more fluid, and in 45 seconds another egg appeared and was thrown in less than 30 seconds. Nothing else was observed to pass during the next half hour. Practically all oviposition observed has taken place while the fleas have been attached to the host. The adults remained attached on the host from 4 to 19 days in the same place. It was not determined whether they reattach after once dropping.

Copulation has been observed to take place on the host. The fleas were attached on the head of the chicken about the length of the body apart and were ventrally by slightly laterally together. They were in

copula when observed and remained so for 35 minutes, until they were slightly disturbed and the act ended.

The adults are killed by freezing temperature and die within a few hours when exposed in tubes in an incubator at a temperature of 100° F.

TOTAL PERIOD OF DEVELOPMENT, LONGEVITY OF ADULT FLEAS, AND CONDITIONS AFFECTING ABUNDANCE

The minimum period from oviposition to the emergence of the adult was 30 days and the maximum period 65 days.

Eggs, larvæ, and pupæ were kept in a jar with the original breeding media from December 8 until April 18, when some adults were still alive after a period of 132 days. The adults can live for a considerable time without food under some conditions, and an infestation will persist for at least two months in the adult stage if the weather is dry and cool. Adults die very quickly without food if the weather is hot.

Breeding occurred during the winter of 1921–22, at Uvalde, Tex., in unprotected places where a large number of chickens were kept—that is, in open yards and under trees in which the fowls roosted. These infestations increased during the winter and early spring until they were very heavy in the latter part of March. The fleas practically disappeared immediately after the heavy rains of March 29 and April 3 and 4. Two weeks after the rains it was unusual to find a flea on the fowls in such places if the latter were not permitted to go under buildings. It has been observed that the fleas become abundant during the fall or spring of the year when the weather is cloudy and dry, a condition that occurs frequently in southwestern Texas. Infestations are likely to occur at any time in cool, dry places, as under buildings and in closely constructed henhouses.

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